Dispersal and Genetic Variation in a Naturally Fragmented Environment: Insights from a Long-term Study of White-throated Dippers (*Cinclus cinclus*)

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Dispersal and Genetic Variation in a Naturally Fragmented Environment: Insights from a Long-term Study of White-throated Dippers (*Cinclus cinclus*)

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Contents

Summary

Many species live in fragmented environments because suitable habitat is often discontinuous, either naturally or due to increasing anthropogenic activities. With populations becoming smaller and more isolated from each other, they have an increased risk of extinction. This highlights the importance of studies on species living in fragmented environments, not least because these include many species of conservation concern. In the first chapter, I introduce relevant aspects in the biology of species living in such environments. The white-throated dipper (*Cinclus cinclus*) is a bird species that lives along rivers and is therefore ideal to study important aspects of dispersal behaviour and genetic variation in a naturally fragmented environment. For this thesis, which is structured into four data chapters, I used data of a long-term study from this species in the proximity of Zurich (Switzerland).

Dispersal is one of the most important life-history traits, being of relevance for many ecological and evolutionary processes. It is often described in terms of distances and rates. However, we still know little about the spatiotemporal properties of the dispersal process itself due to the difficulties of following single individuals over extended periods of time. Based on two years of weekly mark-resight data from one river, I illustrate emigration from the natal site, a highly mobile transient phase, and the process of settlement, as well as patterns of temporary emigration from the natal river (Chapter 2). I conclude that exploratory behaviour during the transient phase is important for finding territories and mates, irrespective of whether individuals settle in their natal or a different population. My findings suggest that movement data can also be valuable for studies on settlement and mate choice as they allow specifying visited territories and narrowing down potential mates.

The avoidance of inbreeding, i.e. mating among relatives, is suggested to be one of the ultimate causes for the evolution of dispersal. Understanding the evolutionary link between dispersal, inbreeding and its avoidance requires data on dispersal and how it shapes the occurrence of inbreeding (Chapter 3). I show that dispersal in dippers is female-biased and often over short distances, but nearly half of all individuals disperse among rivers. In line with this, genetic (microsatellite) data revealed only weak genetic differentiation among rivers, even on a large spatial scale, but substantial levels of genetic structure within rivers. Inbreeding occurred frequently due to small population sizes and the linear habitat structure. Furthermore, females had higher probabilities of inbreeding than males, if they were breeding in their natal river. This difference is likely to contribute to the maintenance of the observed female-biased dispersal behaviour. In conclusion, I argue that weak genetic differentiation among populations does not

exclude the frequent occurrence of inbreeding within populations, in particular in small populations of species living in fragmented habitats.

The consequences of inbreeding, i.e. inbreeding depression, are usually quantified by regressing individual phenotypic values against inbreeding coefficients, implicitly assuming there is no correlation between phenotypes and relatedness of mates. However, taking wing length as an example (Chapter 4), I showed that during part of the study period, parents of inbred birds had shorter wings than those of outbred birds. Because wing length is heritable, inbred individuals were smaller, independent of any inbreeding effects. This resulted in the overestimation of inbreeding effects if additive genetic effects were not accounted for. Similarly, during a different period when parents of inbred birds had longer wings, I found that inbreeding effects were underestimated. I highlight the importance of simultaneously accounting for inbreeding and additive genetic effects because phenotype-associated inbreeding is likely to also occur in other systems, e.g. due to phenotype-dependent dispersal in fragmented environments. In this chapter, I demonstrate how unbiased estimates of inbreeding depression can be obtained within a quantitative genetic framework.

Information on the heritability of quantitative traits is not only of relevance due to the potential influence on estimates of inbreeding depression, but also for predicting responses to selection. Telomere length is an example for an important quantitative trait that has been shown to be linked to fitness-related parameters in a number of species. However, whether variation in telomere length is transmitted from one generation to the next in the wild, and if it is, by which mechanism, is still elusive. Using measures of early-life telomere length in dippers (Chapter 5), I showed that additive genetic variance and heritability were close to zero. Nevertheless, mother and offspring (and mother and son in particular), as well as offspring sharing the same nest and offspring of the same cohort resembled each other due to non-genetic maternal and common environment effects. I conclude that non-genetic environmental factors are the main drivers of variation in early-life telomere length in dippers, which will severely bias estimates of heritability when not modeled explicitly. Given that telomere dynamics are known to be modulated to a large extent by environmental factors also in other species, heritability is likely to be low in the wild, allowing only for weak response to selection.

In summary, I used various approaches to obtain new insights into aspects of dispersal and genetic variation in fragmented environments, based on a long-term individual-based data set of white-throated dippers. In the last chapter (Chapter 6), I summarize my insights that have been provided by four years of work and outline how future research can contribute to our understanding of the biology of species living in fragmented environments.

Zusammenfassung

Viele Tier- und Pflanzenarten kommen heutzutage in räumlich fragmentierten Lebensräumen vor, da geeignetes Habitat auf Grund natürlicher Gegebenheiten und menschlicher Einflüsse nicht weiträumig zur Verfügung steht. Werden Populationen kleiner und sind räumlich isolierter, so steigt gleichzeitig das Risiko, dass sie aussterben. Wissenschaftliche Studien an Arten, die in fragmentierten Lebensräumen vorkommen, sind daher von hoher Relevanz, insbesondere da sich viele bedrohte Arten unter ihnen finden. Im ersten Kapitel dieser Arbeit führe ich in wichtige Aspekte zur Biologie von Arten, die in solchen Lebensräumen vorkommen, ein. Die Wasseramsel (*Cinclus cinclus*) ist eine Vogelart, die entlang von Flüssläufen vorkommt, und sich daher ideal für Studien zum Dispersal-Verhalten und zur genetischen Variabilität in natürlich fragmentierten Landschaften eignet. Für meine Arbeit greife ich auf Daten aus einer Langzeitstudie über diese Art in der Nähe von Zürich (Schweiz) zurück.

Dispersal, die Abwanderung vom Geburtsort (oder vom Ort der eigenen Reproduktion), ist ein wichtiges Verhalten, das für viele ökologische und evolutionäre Vorgänge von Relevanz ist. Die dabei zurückgelegten Distanzen bzw. der Anteil wandernder Individuen dienen häufig seiner Beschreibung. Jedoch wissen wir bislang erst wenig über den genauen zeitlich-räumlichen Ablauf dieser Wanderung, was häufig mit der Schwierigkeit verbunden ist, einzelne Tiere über längere Zeiträume zu verfolgen. Basierend auf wöchentlichen Beobachtungen individuell markierter Wasseramseln entlang eines Flusses über einen Zeitraum von zwei Jahren zeige ich die Abwanderung vom Geburtsort, eine Zwischenphase mit hoher Mobilität, und den Prozess der Ansiedlung (Kapitel 2). Auch Zeichen von vorübergehender Abwanderung sind deutlich zu erkennen. Ich schlussfolgere, dass diese weniger stationäre Phase dem Erkunden möglicher Territorien und Paarungspartner dient, unabhängig davon, ob sich der Vogel letztendlich im Geburts- oder in einem anderen Fluss ansiedelt. Daten mit zeitlich-räumlicher Auflösung könnten somit auch für Studien zu Ansiedlungsverhalten und Partnerwahl von Wert sein, da sie die besuchten Territorien und mögliche Partner einzugrenzen erlauben.

Die Vermeidung von Inzucht, d.h. die Verpaarung unter Verwandten, wird als einer der Gründe für die Entstehung von Dispersal betrachtet. Um die Zusammenhänge zwischen Dispersal, Inzucht und deren Vermeidung zu verstehen, benötigen wir Informationen zum Dispersal-Verhalten und darüber, wie es das Auftreten von Inzucht beeinflusst. Im Kapitel 3 zeige ich, dass Dispersal bei Wasseramseln häufig über kurze Distanzen erfolgt, insbesondere bei Männchen. Etwa die Hälfte aller Individuen, mehr Weibchen als Männchen, siedeln sich aber nicht im Geburtsfluss an. Dies ist im Einklang mit genetischen Daten (Mikrosatelliten), die nur geringe genetische Unterschiede

zwischen Wasseramseln aus verschiedenen Flüssen zeigen, selbst über grössere räumliche Distanzen. Innerhalb eines Flusses können sich hingegen räumliche genetische Muster bilden. Bedingt durch die relativ kleine Anzahl von Vögeln innerhalb eines Flusses und die lineare Habitatstruktur kommt es häufig zu Inzucht. Dabei haben Weibchen eine höhere Wahrscheinlichkeit als Männchen sich mit einem Verwandten zu verpaaren, wenn sie sich im Geburtsfluss ansiedeln und insbesondere wenn dies nahe am Geburtsort geschieht. Dieser Unterschied zwischen den Geschlechtern trägt wahrscheinlich auch zur Beibehaltung der Unterschiede im Dispersal-Verhalten zwischen Männchen und Weibchen bei. Zusammenfassend zeigt dieses Kapitel, dass Inzucht trotz geringer genetischer Differenzierung zwischen Populationen relativ häufig auftreten kann, insbesondere in kleinen Populationen, die in räumlich fragmentierten Lebensräumen vorkommen.

Die Folgen von Inzucht, bezeichnet als Inzuchtdepression, werden üblicherweise mittels Regression von Phänotypen auf die jeweiligen Inzuchtkoeffizienten quantifiziert. Dies erfolgt unter der Annahme, dass keine Korrelation zwischen den Phänotypen (z.B. der Grösse oder der Lebensdauer) und der Verwandtschaft zweier Partner besteht. Nimmt man jedoch die Flügellänge bei Wasseramseln als Beispiel (Kapitel 4), stellt sich heraus, dass über einen gewissen Zeitraum der Studie miteinander verwandte Partner (d.h. Eltern von ingezüchteten Individuen) kürzere Flügel hatten als unverwandte Partner. Da Flügellänge vererblich ist, waren ingezüchtete Individuen kurzflügeliger, und zwar unabhängig von Inzuchtseffekten. Blieben additiv genetische Effekte in der Analyse unberücksichtigt, so führte dies zu einer Überschätzung der Inzuchtseffekte. Über einen anderen Zeitraum der Studie waren Eltern ingezüchteter Vögel jedoch langflügeliger, was entsprechend zu einer Unterschätzung der Inzuchtseffekte führte. Mit diesem Kapitel zeige ich, dass es wichtig ist gleichzeitig additiv genetische und Inzuchtseffekte zu berücksichtigen. Dies ist insbesondere von Bedeutung, da das Auftreten von Inzucht auch in anderen Arten mit dem Phänotyp eines Merkmals korreliert sein kann, z.B. wenn das Dispersal-Verhalten mit diesem Merkmal assoziiert ist. Ich zeige, wie Inzuchtdepression präzise mittels quantitativ genetischer Methoden gemessen werden kann.

Informationen über die Erblichkeit eines Merkmals sind nicht nur von Relevanz, weil Erblichkeit Messungen von Inzuchtdepression verfälschen kann, sondern auch da die Erblichkeit die Möglichkeiten aufzeigt, wie ein Merkmal auf Selektion reagieren kann. Die Länge von Telomeren ist ein Beispiel für ein wichtiges Merkmal, dessen Bedeutung für die Fitness bereits in verschiedenen Arten gezeigt wurde. Jedoch ist noch relativ unbekannt, ob die Variation in diesem Merkmal von einer Generation auf die nächste weitergegeben wird, und wenn ja, über welchen Mechanismus. Basierend auf Messungen von Telomeren in jungen Wasseramseln zeige ich in

Kapitel 5, dass sowohl additiv genetische Varianz als auch Erblichkeit nahe Null lagen. Trotzdem ähnelten sich die Telomerlängen von Müttern und ihren Nachkommen (insbesondere ihrer Söhne), sowie von Jungvögel im gleichen Nest sowie aus dem gleichen Jahr auf Grund nicht-genetischer maternaler Effekte bzw. einer gemeinsamen Umgebung. Ich schlussfolgere, dass nicht-genetische Effekte die Variabilität in der Länge von Telomeren massgeblich beeinflussen und sie deshalb auch in Studien zur Erblichkeit dieses Merkmals Berücksichtigung finden sollten. Da auch in anderen Arten ein starker Einfluss von Umweltfaktoren gezeigt werden konnte, ist die Erblichkeit in natürlichen Populationen vermutlich häufig klein, sodass Selektion nur geringe Veränderungen in diesem Merkmal auslösen kann.

Zusammenfassend habe ich verschiedene methodische Ansätze verfolgt, um neue Einsichten in verschiedene Aspekte von Dispersal und genetischer Variation in räumlich fragmentierten Lebensräumen zu gewinnen. Im letzten Kapitel fasse ich diese Erkenntnisse aus meiner vierjährigen Arbeit zusammen und zeige Fragestellungen für zukünftige Forschung auf, die unser Verständnis der Biologie von Arten in fragmentierten Lebensräumen erweitern können.

Chapter 1

General introduction

From continuous to fragmented environments

(Ahlroth et al. 2010)Species of plants and animals typically have a number of requirements towards their environment. They usually prefer a certain type of habitat that allows them to survive and reproduce. In some cases, suitable habitat occurs continuously over large spatial scales. However, often habitat is spatially discontinuous. This habitat fragmentation can be of natural origin, as is the case for islands, mountaintops, and water bodies like lakes, ponds or rivers. Although single rivers might be connected within a river system at a large scale, a species' distribution in a riverine environment can be discontinuous and thus fragmented, as the river habitat is not suitable throughout (Shipham et al. 2013). Other habitats like forests or marshes became fragmented due to far-reaching human activities, especially in the last few centuries. With species' distributions getting more and more scattered and habitat fragments becoming smaller, the risk of local extinctions increases substantially (Smith and Keller 2006). A good understanding of the biology of small and fragmented populations is therefore of prime importance for the persistence of many species, especially of those already being endangered.

If individuals are able to move from one fragment to another, they can sustain small populations or even (re-)colonize empty habitat fragments (Hanski 1998). However, whether they are able to do so depends not only on the geographical distance between two habitat fragments but also on the interspersed habitat matrix. For example, high mountains, deserts or huge water bodies, but even forests, rivers or agricultural land can display ecological barriers. Species differ considerably in their abilities to move over short or long distances and in what they perceive as barriers. The movement of individuals that connects populations in different habitat fragments is one possible and important outcome of a behaviour that is termed dispersal.

Dispersal connects populations

Dispersal is defined as the movement of individuals between the sites of birth and first breeding (natal dispersal), or of successive breeding events (breeding dispersal) (Greenwood and Harvey 1982). Typically, natal dispersal is the main movement of individuals when compared to breeding dispersal (Paradis et al. 1998 and references therein). Dispersal can be both between habitat fragments (or populations) and within a habitat fragment (or population). It is typically measured by means of Euclidean distances between sites. The distribution of distances is often visualized as a dispersal kernel, i.e. a probability density function of distances. Often, however, the tail end of this distribution gets underestimated because dispersal over long distances is likely to remain undetected in study areas of limited size (Van Noordwijk 1984, Koenig et al. 1996). Furthermore,

we can estimate the dispersal rate from the number of individuals moving within (termed philopatric individuals) and between (dispersing individuals) habitat fragments (or populations), respectively. Whereas many studies describe dispersal based on such summary statistics, we still know little about the spatiotemporal properties of the dispersal process in free-ranging animals. This is mainly due to the difficulties of following single individuals precisely over extended periods of time.

A large body of both theoretical and empirical work on dispersal (Clobert et al. 2001, Clobert et al. 2012) has identified multiple causes that influence individual dispersal behaviour. As also suggested by theory, population density and habitat quality have been found to affect dispersal (Harrison 1980, Verhulst et al. 1997, Matthysen 2005, Pärn et al. 2012). While individuals are expected to avoid low quality habitat or high densities (but see Allee effect, Stephens et al. 1999) they might differ in both their tolerance of adverse conditions and in other traits that make them more or less likely to disperse. For example in birds and many insects, dispersal behaviour is a function of body size, especially wing length, with bigger or longer winged individuals dispersing further (Paradis et al. 1998, Skjelseth et al. 2007, Dawideit et al. 2009, but see Chaput-Bardy et al. 2010). Furthermore, philopatric and dispersing individuals have been shown to differ in behavioural and life-history traits, like boldness, survival or reproductive success (Innocent et al. 2010, Cote et al. 2011). Consequently, dispersal behaviour is not only variable between species (e.g. Paradis et al. 1998) but also between individuals of the same species, suggesting that dispersing individuals are not a random subsample of the population.

Quantitative traits such as dispersal behaviour are typically shaped by many genes and the environment. Studies showing that dispersal is condition-dependent, i.e. is triggered by external factors like population density or habitat quality to a large extent, suggest that there is no or only little genetic variation of dispersal behaviour (Ims and Hjermann 2001). In contrast, other studies found evidence for sometimes considerable genetic variation underlying dispersal behaviour (Roff and Fairbairn 2001, Hansson et al. 2003, Doligez et al. 2009, Korsten et al. 2013). This, in turn, would allow dispersal to evolve in response to selection (Thomas et al. 2001).

Consequences of dispersal

As already mentioned before, dispersal has a major implication on various ecological and evolutionary levels (Clobert et al. 2012). Depending on the balance between emigration and immigration (i.e. dispersal away or into a population), dispersal shapes population dynamics, ranging from (re-) colonization of empty habitat to local extinctions (Hanski 1998). Therefore,

dispersal can be of relevance for changes in species' range margins. For example, range shifts or expansions will be of importance for the survival of species under anthropogenic climate change (Kokko and López-Sepulcre 2006). Given that dispersing individuals are often not a random sample with respect to phenotypes, dispersal also shapes phenotypic variation and might thereby influence adaptation to local environmental conditions (Postma and Van Noordwijk 2005, Benton and Bowler 2012). When dispersal results in the movement of genes (i.e. gene flow), it alters the genetic composition both within and between populations (see below). When gene flow is restricted between populations, they can diverge genetically and phenotypically due to genetic drift and selection. Eventually, these processes can result in allopatric speciation. Finally, as dispersal brings individuals away from their natal or breeding site, it is likely to reduce both competition and mating with kin. Together with environmental variability (Lurz et al. 1997), the avoidance of kin competition and inbreeding are discussed as the ultimate drivers for the evolution of dispersal behaviour (Hamilton and May 1977, Gandon and Michalakis 2001, Guillaume and Perrin 2006).

Linking dispersal to inbreeding

Inbreeding is defined as the mating among relatives due to either non-random mating or genetic drift. In particular in small and isolated populations it can occur frequently (Lande 1988, Keller and Arcese 1998, Reid et al. 2014). The degree of inbreeding is typically measured as the probability of the two alleles of an individual being identical by descent with respect to a reference population (inbreeding coefficient f, Wright 1922). For example, if parents are full siblings, their offspring have a probability of 25% of being homozygous at any locus because alleles are identical due to common ancestry. Inbreeding coefficients are therefore commonly deduced from pedigree data, which represent the relatedness between individuals (see below). Alternatively, as inbreeding leads to increased homozygosity, inbreeding has been inferred from multi-locus genotype data (e.g. Bolund et al. 2010) (for discussion see Balloux et al. 2004, Slate et al. 2004, Bérénos et al. 2014).

As it increases homozygositiy, inbreeding results in deleterious recessive mutations being expressed with higher probability, a reduction in the frequency of heterozygotes at loci showing overdominance, and/or changes in gene interactions, all of which may negatively affect trait values and fitness (Crow and Kimura 1970, p 78-80). Since Darwin's investigations on the subject (1876), numerous studies testing for negative consequences of inbreeding (i.e. inbreeding depression) have shown that inbreeding depression is common, both in captive and wild

populations (Charlesworth and Charlesworth 1987, Keller and Waller 2002). Inferring inbreeding depression from the relationship between phenotype and inbreeding coefficient assumes that inbreeding individuals are a random subsample of the population with respect to the trait of interest. Although there is abundant evidence for phenotype-associated inbreeding (e.g. Richardson et al. 2004, Reid et al. 2008), its effects on estimating inbreeding depression have so far not been quantified empirically.

In order to avoid inbreeding and its detrimental effects, dispersal has been suggested as a potential mechanism (Szulkin et al. 2013), not requiring additional mechanisms of active avoidance of reproduction with kin (e.g. kin recognition). Tests of inbreeding avoidance can be problematic as they require adequate null models that need to specify potential mates based on various assumptions (e.g. Pärt 1996, Szulkin et al. 2009). Furthermore, empirical studies testing how dispersal, and in particular sex-biased dispersal, influences the probability of inbreeding are scarce and with contradicting conclusions (Pärt 1996, Eikenaar et al. 2008, Szulkin et al. 2009, Lebigre et al. 2010).

Dispersal shapes spatial genetic variation

If dispersal results in gene flow, it also shapes genetic variation within and between populations. As gene flow is typically limited within the species range, neighbouring individuals are genetically more similar than distant individuals (isolation by distance, see Wright 1943, Malécot 1948, Watts et al. 2007). On these grounds, genetic data have been used to obtain indirect estimates of dispersal (e.g. Watts et al. 2007, Selonen and Hanski 2010). On the population level, genetic drift can lead to genetic differentiation, which can be quantified with Wright's F-statistics (see Whitlock and McCauley 1999) and a number of related measures (Meirmans and Hedrick 2011, Whitlock 2011). For example, restricted gene flow was found to generate genetic structure even over small spatial scales (e.g. Postma et al. 2009, Athrey et al. 2012, Banks and Peakall 2012). In other cases, genetic differentiation is low or even absent over large spatial scales due to high levels of gene flow (Postma et al. 2009, Kekkonen et al. 2011). Very often, microsatellite markers are used to illustrate the spatial patterns of genetic variation. They are characterized by their high degree of polymorphism, their codominant inheritance, and their putative neutrality. However, gene flow through dispersal does not only shape neutral but also quantitative genetic variation (i.e. genetic variation underlying quantitative traits). In particular the heritable part of quantitative genetic variation is of importance for understanding and predicting the response of phenotypic variation to selection.

Heritable genetic variation

Quantitative traits are shaped by typically a large number of genes and the environment. While environmental conditions are kept constant in laboratory settings, they are usually highly variable and thus of significance in natural environments. Quantitative genetics theory has been developed to analyse phenotypic variation in quantitative traits (Lynch and Walsh 1998). It aims at separating the total phenotypic variance (V_P) in a population into genetic (V_G) and non-genetic, i.e. environmental (V_E), variance components. Assuming independence of V_G and V_E, V_G can be further subdivided into variance due to additive, dominance, and epistatic effects (V_A, V_D, and V_I, respectively). Additive genetic variance estimates the heritable part of genetic variation. The ratio V_A/V_P is known as the narrow-sense heritability (h^2) in a trait and can be used to predict the response to selection (Lynch and Walsh 1998). In practice, heritability can for example be estimated from a regression of offspring on parent phenotypic values, with the slope of the regression being half of the heritability in the trait (Lynch and Walsh, chapter 17). However, these estimates can be confounded by non-genetic causes of resemblance (like maternal effects). The separation of different variance components is possible within a quantitative genetics framework, the so-called animal model (Kruuk 2004). Using restricted maximum likelihood or Bayesian methods (Wilson et al. 2010), various components of variance, for example due to additive genetic effects (animal effect), parental effects or a shared environment can be estimated while accounting for confounding covariates (see Wilson 2008 for a discussion about the interpretation of heritability estimates). Estimates of heritability are important to predict how traits can respond to selection, e.g. due to environmental change. Therefore, studies on fitness-related traits are especially exciting and important. In order to estimate the mentioned variance components, and thus heritability, the animal model requires multigenerational data of phenotypes and relatedness. Such data can be obtained from individual-based long-term studies (see next paragraph).

Long-term studies

In individual-based long-term studies individually recognizable animals are monitored throughout their lives. Such studies produce highly valuable data sets that allow answering a diverse range of questions in ecology and evolution (reviewed in Clutton-Brock and Sheldon 2010). Starting in the first half of the 20th century, longitudinal studies on various species of birds and mammals have contributed to our understanding of, for example, population dynamics, age- and social structure within populations, or natural and sexual selection. Following marked individuals throughout their lives provides information not only on their morphology but also on their behaviour (e.g. dispersal

behaviour or social status) and their reproductive success. Finally, data from a large number of individuals over many years can reveal insights into the biological consequences of environmental change, e.g. on demography or phenotypic variation. For example, a good understanding of the relative contributions of genes and the environment on phenotypes is essential for predicting the response of populations to environmental change.

A long-term study on white-throated dippers

The white-throated dipper (*Cinclus cinclus*) is a medium-sized passerine in the family Cinclidae, which comprises only five species worldwide (Creutz 2009). It lives along streams and rivers and is widely distributed across Europe and parts of Asia and north-western Africa. The subspecies *C. c. aquaticus* occurs in Central and Southern Europe in suitable riverine habitat in hilly or mountainous environments. Provided with several morphological adaptations that allow dippers to swim and dive even in fast-flowing rivers, they mainly feed on aquatic invertebrates.

Since 1987, Johann Hegelbach and his students have been monitoring dippers intensively at up to eleven rivers, spanning an area of approximately 20 x 20km at altitudes between 400 and 680m a.s.l in the proximity of Zurich, Switzerland (8°23'E / 47°25'N to 8°40'E / 47°10'N, Fig. 1). In this part of the Swiss midlands, basically all dippers are resident, meaning that they can be observed year-round.

Early in the year, dippers occupy territories of several hundred meters in length. Whereas most pairs are socially monogamous, each year approximately 9% of males are paired with two females in our study area. The round nest out of moss, blades of grass and leaves is always close to the water. It is built into a crack or a hollow in the masonry or the rock, also behind waterfalls, on the supports of bridges, or, although rarely, in overhanging branches and roots. However, they also accept nest boxes for breeding. Females typically lay between four and maximally six eggs, and incubate them for 16-17 days. In our study area, offspring of the first brood hatch between the middle of March and the beginning of May. About 35% of all offspring are from second broods (Hegelbach 2013) hatching between the end of April and the beginning of June. Both parents provide parental care before offspring fledge with 21-24 days (Schoop 1997).

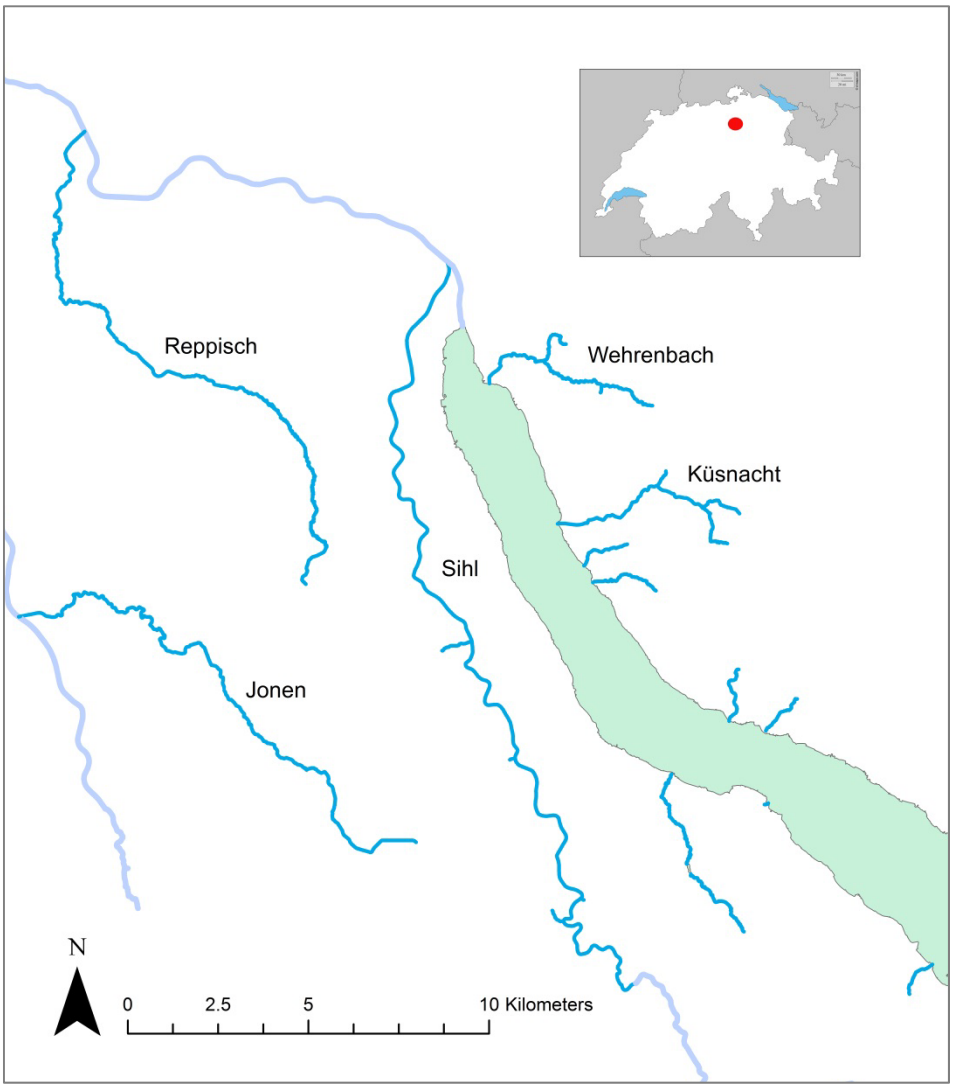

Figure 1: Map of the study area around the Lake of Zurich (Northern Switzerland). The city of Zurich is located at the northern edge of the lake. The eleven study rivers are coloured in blue. Five of them, to which this thesis refers explicitly, are labelled. Whereas the rivers in the east discharge into the Lake of Zurich, the Sihl, Reppisch and Jonen rivers discharge into the larger Limmat and Reuss rivers (in grey), respectively. The upper part of the Sihl river, which is not monitored, is coloured grey as well. [Map from swisstopo, www.swisstopo.ch, modified with ArcMap 10.0 (ESRI)]. Inset: Map of Switzerland with the position of the study area marked as red circle.

Monitoring started in 1987 at the two rivers Küsnacht and Wehrenbach (Fig. 1 and 2), which are suitable for dippers over 8.0 and 7.0km including smaller side rivers, respectively. They both mouth into the Lake of Zurich, with a discharge of normally around 0.5 and 0.2m^3/s and a drainage basin of approximately 13 and 9km^2, respectively (Hegelbach 2013). Monitoring in a third river, the Sihl (Fig. 1 and 2), started in 1990. This river has been monitored over the last 25.5km before it joins with the larger Limmat river in the city of Zurich. Due to the large size of its drainage basin (2400km^2) the Sihl river is 20-30m broad but shallow, and has a drainage volume of at least 2.5m^3/s throughout the year.

Two further rivers (Reppisch and Jonen with 20.5 and 17.5km, respectively) are located west of the Lake of Zurich and discharge into the larger Limmat and Reuss rivers. They are monitored since 1997 and 2001, respectively, but every year some occupied territories may have been missed. The remaining rivers within the study area all discharge into the Lake of Zurich, but harbour only very small populations (<5 pairs).

Figure 2: Wehrenbach (top), Küsnacht (middle), and Sihl (bottom).

Every year, field work to map territories starts in January in order to find nests in due time. On average, approximately 80 territories (including a mean of 13, 7 and 27 territories in the Küsnacht, Wehrenbach and Sihl rivers, respectively) are checked regularly between nest building and nestling phase. When nestlings are 10-14 days old (min. 7 days, max 16 days), they are ringed, measured and since 2001 a small blood sample (max. 30 µl) is collected by puncturing the tarsal vein in some rivers. All nestlings are ringed with metal rings of the Swiss Ornithological Institute. Independent and fully-grown individuals are recaptured and equipped with two colour rings to also allow for identification using binoculars or a telescope. Unringed adults (i.e. immigrants) are captured using mist nets and colour-ringed, measured and blood sampled, usually before the breeding season, but at the latest before their offspring are ringed. Due to the high intensity of field work, virtually all parental individuals are known in these rivers (between 1996 and 2013 only 0.1% and 0.5% of ringed nestlings have an unknown mother or father, respectively) and less than 1% of all broods was inaccessible. Fieldwork procedures are licensed by the Swiss Federal Office for the Environment and the Veterinary Office of the Canton of Zurich.

Parentage of each brood was determined from behavioural observations, assuming that the social parents are also the genetic parents of a nestling. This is a reasonable assumption given the low incidence of extra-pair paternity in these study populations (2% according to Øigarden et al. 2010; less than 1% according to our own unpublished data). Using 7682 offspring that have been ringed between 1987 and 2013 and their parents, we were able to construct a pedigree spanning up to 15 generations (Fig. 3).

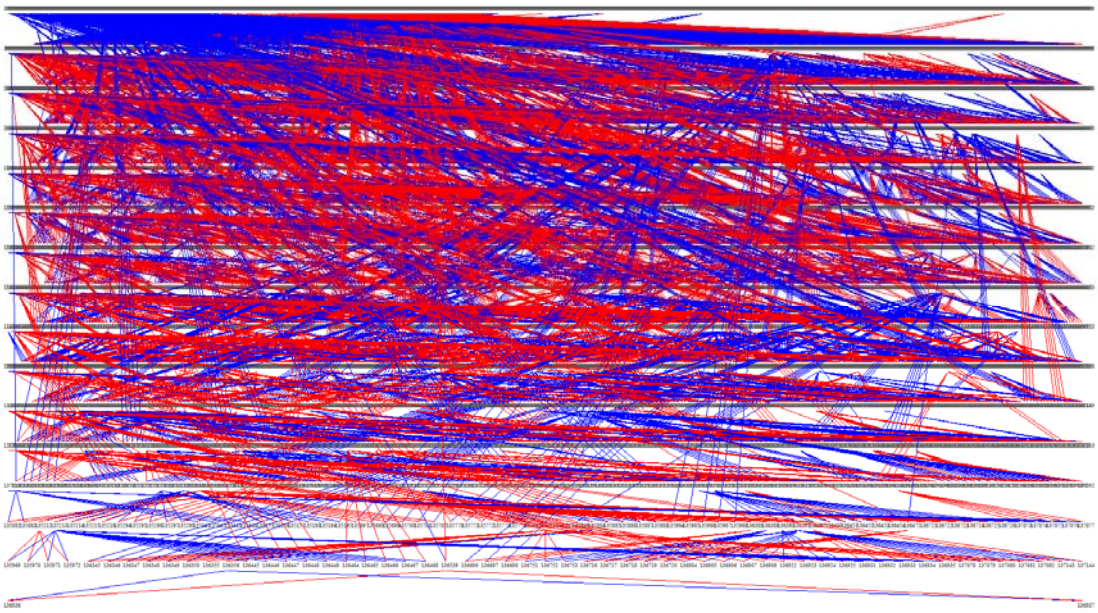

Figure 3: Representation of the pedigree of dippers in the Zurich study area. Red and blue lines connect mothers and fathers with their offspring, respectively. The pedigree spans a maximum of 15 generations and is arranged by pedigree depth. The figure was produced using the software *Pedigree Viewer*.

Outline of this thesis

This thesis, which is structured into four data chapters (Chapters 2-5), uses the described long-term study on white-throated dippers in Northern Switzerland to contribute new insights to our knowledge of dispersal, genetic variation and inbreeding in a naturally fragmented environment.

As has been summarized above, there is a large body of both theoretical and empirical work on dispersal behaviour. However, we still know little about the spatiotemporal properties of the dispersal process itself in free-ranging animals. Animals are likely to adjust their behaviour in response to various internal and external factors and show complex movement patterns. In many cases, however, following single individuals precisely and intensely over an extended time period is impossible. In contrast, colour-ringed dippers can easily be detected and identified throughout the year owing to the linear habitat and the strong association of dippers with rivers. Using weekly resighting data collected over a period of two years, I studied spatial and temporal patterns of fine-scale movements. In particular, I quantified within-river movements away from the natal site (juveniles) or the previous breeding site (adults) and towards the future breeding site, as well as towards an individual's future partner. Subsequently, I tested for temporary emigration from the study river using Bayesian multi-state mark-recapture models. Using this unique data set, I present insights into the dispersal process in general, and into aspects of settlement and mate choice in particular in Chapter 2.

Dispersal has been suggested to have evolved as a mechanism to avoid inbreeding that does not require the active avoidance of relatives during mate choice (Pusey and Wolf 1996, Gandon and Michalakis 2001). Sex-biased dispersal alone might already reduce the probability of inbreeding (Eikenaar et al. 2008, Lebigre et al. 2010). Understanding the effect of dispersal on the occurrence of inbreeding requires a comprehensive picture of individual dispersal behaviour and its outcome in terms of gene flow. In chapter 3, I first describe dispersal patterns in a naturally fragmented environment, using both data from the long-term study and ring recovery data of birds, which have been ringed in the course of the study and reported to the Swiss Ornithological Institute. In addition, I quantified the effects of dispersal on spatial genetic variation using microsatellite data. Finally, I used information from the multi-generational pedigree (Fig. 3) to quantify levels of inbreeding, and relate patterns of inbreeding to dispersal behaviour, and to sex-biased dispersal in particular. In this chapter, I highlight that understanding the consequences of dispersal on the occurrence of inbreeding in fragmented environments is of high importance, as an increasing number of species lives in fragmented and small populations, including many species of conservation concern.

In particular in small and fragmented populations and when dispersal is limited, inbreeding can occur frequently. In order to quantify the effects of inbreeding, individual phenotypic values are typically regressed on inbreeding coefficients, while accounting for confounding covariates like age, sex or year. This standard method assumes that inbreeding individuals are a random subsample of the population with respect to the trait of interest. In other words, it assumes that there is no correlation between an individual's phenotype and the kinship coefficient to its mate, i.e. the inbreeding coefficient of their offspring. However, if, for example, related mates are characterized by lower trait values than unrelated ones, inbred offspring will have parents with lower trait values. In chapter 4, I show that such non-random patterns can lead to both under- and overestimation of the inbreeding effect when additive genetic effects are not accounted for, using wing length as an example. I highlight the importance of simultaneously accounting for inbreeding and additive genetic effects to obtain unbiased estimates of inbreeding depression and demonstrate how this can be done within a quantitative genetic framework.

In the previous chapter, I focussed on the effect of inbreeding, while accounting for the heritability in a trait. In chapter 5, I study patterns of inheritance while accounting for inbreeding effects. Thereto, I use telomere length as a trait that has been shown to predict life-history parameters (e.g. lifespan) in various species, making telomere length a trait of particular relevance in ecology and evolution (e.g. Bize et al. 2009, Heidinger et al. 2012). Telomeres are protective DNA-protein complexes located at the ends of eukaryotic chromosomes. Although the link to fitness-related traits suggests that telomere length is subject to natural selection, its evolutionary dynamics crucially depends on its heritability. In chapter 5, I test whether and how variation in early-life relative telomere length (measured as the amount of telomere sequences relative to a control gene using quantitative polymerase chain reaction, qPCR) is transmitted across generations, using DNA samples and pedigree information from our long-term data set. Within a quantitative genetics framework I disentangle the relative effects of genes and environment, and test for sex-specific patterns of inheritance.

In a final chapter, I summarize the insights on dispersal and genetic variation in fragmented environments that were made possible by four years of work on a long-term data set of a fascinating bird species, and provide perspectives for future research.

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Chapter 2

Dispersal is more than going from A to B: fine-scale movement patterns and temporary emigration in white-throated dippers

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Abstract

Although dispersal is among the most important life-history traits, data about the process of dispersal in free-ranging animals are still scarce. Here, we use two years of weekly mark-resight data of colour-ringed white-throated dippers (*Cinclus cinclus*), a non-migratory bird species living exclusively along rivers, to quantify spatial and temporal patterns of fine-scale movements within a single river, and to test for temporary emigration. We show that whereas adult dippers remain in the proximity of their breeding site throughout the year, juveniles are much more mobile, with the distance from the natal territory increasing rapidly few weeks after fledging. Furthermore, we show how such mark-resight data can reveal additional insights into the processes of mate choice and settlement. Using a multistate mark-recapture model, we estimated patterns of temporary emigration and immigration as well as apparent survival. Juveniles of both sexes and adult males were less likely to be present in the study river between breeding seasons due to temporary emigration. On the whole, we conclude that the high mobility in juveniles, including their temporary emigration, is an important component of dispersal behaviour. It enables juveniles to explore adjacent habitat and increase familiarity over a larger area, allowing for informed dispersal.

Keywords: bird · dispersal · exploratory behaviour · temporary emigration · movement

Introduction

Dispersal, the movement between the site of birth and the site of first breeding (natal dispersal) or between successive breeding sites (breeding dispersal), is often described as a process consisting of three stages, which are emigration, transience and immigration (Clobert et al. 2012). However, in truly migratory species (i.e. with regular seasonal migration), dispersal is the outcome of a much more complex movement pattern than this three-stage process. Then, dispersal distance measured as the distance between the locations of birth and breeding, can make up only a very small proportion of the lifetime track (Nathan et al. 2008). Also in non-migratory species, individuals may cover substantial amounts of terrain before deciding on a breeding site. Even if they remain philopatric, i.e. breed in their natal population, they may temporarily leave their population. In theoretical dispersal models, movement paths are assumed to be simply governed by diffusion algorithms, more or less strongly correlated random walks or area-restricted search (Levin et al. 2003, Dias et al. 2009). However, free-ranging animals are likely to show much more complex patterns due to their perceptual abilities, allowing them to collect information about the surrounding landscape and their conspecifics and to adjust their behaviour accordingly (e.g. Reed et al. 1999, Getz and Saltz 2008).

While there is a mechanistic framework to describe passive dispersal, e.g. wind-mediated seed dispersal (Kuparinen 2006, Schurr 2012), we still know little about the spatio-temporal properties of the dispersal process in free-ranging animals, and how this is shaped by their environment. In many cases, this is due to the inability to follow single individuals precisely and intensely over an extended time period. Advances in modern tracking technologies (e.g. using radio telemetry or GPS loggers; Naef-Daenzer 2013, Kissling et al. 2014) allow us to track organisms on a much higher spatial and temporal resolution. Yet, they require intense tracking to enable following emigrating individuals (e.g. under radio telemetry) or a certain minimum body mass for attaching data loggers (e.g. satellite loggers, Aebischer et al. 2010). Finally, tracking may be complicated because the species lives in an aquatic environment (Lowther et al. 2013). Alternatively, marked individuals can be followed through routine census or capture efforts in one or several patches or populations. Accounting for imperfect detection, such resight or recapture data can likewise be used for the reconstruction of movement paths.

When dispersal occurs between habitat patches or populations in a fragmented environment, this involves emigration from the monitored population. When using recapture or resight data from this population only, such permanent emigration cannot be disentangled from mortality because both states are virtually unobservable. Permanent emigration will therefore deflate estimates of survival (Lebreton et al. 1992). However, while some emigrating individuals leave the local

population permanently, other individuals might return at a later point in time. These non-permanent movements away from the local site, termed temporary emigration (Kendall et al. 1997, Schaub et al. 2004), encompass a variety of behaviours and can be applied to both plants and animals. For example, animals like seals, colonial birds or amphibians have been shown to be absent from the breeding area for certain periods of time and to skip breeding seasons (e.g. Muths et al. 2006, Sanz-Aguilar et al. 2011, Stauffer et al. 2013), and if presence and absence are recorded on a finer temporal scale, extended foraging trips may be considered as temporary emigration as well (Lunn et al. 1994). Finally, small mammals may escape detection through torpor, even though being present on the local site. Similarly, annual underground dormancy in plants (Kéry et al. 2005) will generate presence-absence patterns similar to temporary emigration.

Both individual-based tracking data and mark-recapture data (or likewise mark-resight data) can provide us with important information on the intensity, direction and timing of movement, albeit at different temporal resolutions. They can also improve our understanding of vital demographic rates, like dispersal or temporary emigration, including their temporal and spatial features. Whereas many studies focus on dispersal among habitat fragments or populations, knowledge on the dispersal process within a habitat fragment is of high biological relevance and interest too. Dispersal does not only shape genetic variation and patterns of relatedness, it is also critically important for recolonization following extinction, as well as for changes of species' range margins (Clobert et al. 2012). Although increasing habitat fragmentation is likely to constrain dispersal (Ahlroth et al. 2010), it is vitally important for following optimal environmental conditions in a changing world (Kokko and López-Sepulcre 2006).

Here we use white-throated dippers (*Cinclus cinclus*) as a model for a species living in a spatially structured environment, to study the process of dispersal of philopatric individuals. This bird species lives along streams and rivers and mainly feeds on aquatic invertebrates. Due to the spatial arrangement of suitable rivers, it lives in a naturally fragmented environment with linear habitat structure. The studied subspecies (*C. c. aquaticus*) is known to be resident (Glutz von Blotzheim and Bauer 1988) and dispersal is female-biased with a large fraction of individuals being philopatric, i.e. recruiting in their natal river (see Chapter 3 of this thesis). Owing to the linear habitat and the strong association of dippers with the river, marked individuals can easily be detected and identified, making them ideally suited to study the process of dispersal. Using weekly resighting data for colour-ringed individuals collected over a period of two years, we quantify spatial and temporal patterns of fine-scale movements. In particular, we quantify within-river movements away from the natal site (juveniles) or the previous breeding site (adults) and towards the future breeding site, as well as towards an individual's future partner, and we test for

temporary emigration from the study river. Using this unique data set, we can obtain a fascinating insight into the dispersal process in general, and into aspects of settlement and mate choice in particular.

Material and Methods

Study system

The white-throated dipper is a medium-sized passerine (males are 62.5 ± 3.6g and females 53.7 ± 4.1g in the study population; mean ± s.d.) that is widely distributed across Europe. Since 1987, dippers of the subspecies *C. c. aquaticus* have been monitored intensively at a maximum of eleven rivers spanning an area of approximately 20 x 20km in the proximity of Zurich, Switzerland (8°23'E / 47°25'N to 8°40'E / 47°10'N, see Fig. 1 in Chapter 1, page 20) (Hegelbach 2004). In this part of the Swiss midlands, basically all dippers are resident, meaning that they can be observed year-round. In three rivers (Küsnacht, Sihl and Wehrenbach) virtually all (>99%) of the reproducing adult birds and their offspring are marked with colour rings. Offspring of the first brood (brood size at ringing: 4.4 ± 1.1 nestlings) hatch between the middle of March and the beginning of May. About 35% of all offspring are from second broods (Hegelbach 2013) with 3.7 ± 1.1 nestlings hatching between the end of April and the beginning of June. About 39% of female and 65% of male offspring recruit in their natal river (see Chapter 3).

Fieldwork

In order to follow the movement patterns between birth and the next breeding season or between successive breeding seasons, we monitored a colour-ringed population in one river (Küsnacht, 6.5km and two short confluences of approximately 0.5 and 0.9km length) over a period of two years (18 April 2011 – 3 April 2012 and 2 May 2012 – 28 March 2013). The population was monitored weekly, avoiding adverse weather conditions like heavy rain to maximize detection probabilities. The mean (± sd) time interval between two counts was 7.0 ± 1.7 days, with a total of 98 counts (86 by P.J.J.B, 11 by J.H., and 1 by P. Nietlisbach). During four weeks (in July 2011, March 2012, September 2012, and January 2013), additional monitoring was conducted at each of the three days following the regular weekly monitoring. Assuming the population to be closed during these four days, we independently estimated detection probability at different times during the year from these data for comparison (see below for more details).

Marked dippers could easily be identified on the basis of their individual combination of two colour rings and one metal ring by walking closely along the river. Only records of individuals that could be undoubtedly identified, i.e. without using additional information on the whereabouts of the individual, were used for the analyses. Data were collected between sunrise and approximately noon, alternately walking up- and downstream. On average (± sd), 30.6 ± 8.7

individuals could be identified per occasion. In addition to the presence of an individual, we noted its position within the river (assignment to one out of 136 spatially referenced river marks). For all analyses, individuals were classified as being juvenile (individuals during their entire first year of life) or adult (individuals older than one year) and as female or male. The latter was determined from a blood sample that was taken at first capture of an individual by amplifying the CHD-W and CHD-Z genes using modified versions of the P2 and P8 primers (Griffiths et al. 1998, Hoeck et al. 2009).

Analysis of fine-scale movement patterns

Based on the spatial coordinates of the above mentioned river marks, we calculated the distance between two observations as a river distance (and not as a straight line distance) between observations from two successive weeks. First, we modelled the decision of moving or staying (i.e. observed twice at the same location) as a Bernoulli process including individual identity as a random effect. Second, we modelled the movement process with log-transformed distances as the dependent variable, again fitting individual identity as a random effect. In order to test for group-specific differences in mobility, we included sex and age (see above) as covariates. To account for the possibility that movement behaviour was influenced by the limited length of the river, we fitted the minimum distance to the lower (Lake of Zurich) or upper end of the river as both linear and quadratic term.

Subsequently, we calculated the distance of an individual to its natal (in juveniles) or its breeding site (in adults), respectively, in each week following the breeding season. Due to mortality and permanent emigration, the number of individuals for which this distance could be calculated decreased throughout the year. Likewise, we calculated the distance of an individual to its future breeding site, separately for juveniles (i.e. new recruits) and adults, for all individuals with a breeding attempt in the study river in the next season. In addition, we included the information on whether the individual was observed up- or downstream of its future breeding site. Finally, we calculated the distance between two future mates. Only if both individuals were observed on the same occasion, this distance could be calculated. We here distinguished between whether pairs consisted of two adults, two juveniles or an adult and a juvenile.

Descriptive and statistical analyses were performed using R version 3.0.2 (R Development Core Team 2013). Mixed effect models were run using the package lme4 (Bates et al. 2012).

Multistate mark-recapture model

We used a multistate mark-recapture (or in our case, a mark-resight) model that accounts for temporary emigration (Kendall and Nichols 2002, Schaub et al. 2004). We formulated it as a hierarchical model incorporating a state and an observation process. Each individual can be assigned a certain state as an individual categorical covariate that can change from occasion to occasion, allowing for the estimation of state transition probabilities. The individuals are in one of the three states "present", "absent" or "dead". The state transition matrix describes how individuals move among states from one occasion to the next:

$$\begin{bmatrix} \phi_{t,a,s}\left(1-\varepsilon_{t,a,s}\right) & \phi_{t,a,s}\varepsilon_{t,a,s} & 1-\phi_{t,a,s} \\ \phi_{\varepsilon_{t,a,s}}\omega_{\varepsilon_{t,a,s}} & \phi_{t,a,s}\left(1-\omega_{\varepsilon_{t,a,s}}\right) & 1-\phi_{t,a,s} \\ 0 & 0 & 1 \end{bmatrix}$$

Here, ϕ is the apparent survival probability, ε the probability of temporary emigration, and ω the probability of immigration. The subscripts refer to time (*t*), age class (*a*; as defined above) and sex (*s*). Owing to imperfect detection, we can only observe part of the individuals in the state "present", and the two other states are completely unobservable. Thus, our data only contain fragmentary information about the true states, namely whether or not an individual was observed at a given occasion in the study river. To make inference, we therefore formulated an observation model that is conditional on the state process model. This model can again be described by a transition matrix, but here the transition matrix links the three true states (rows) with the observations (observed vs. unobserved; columns):

$$\begin{bmatrix} p_{t,a,s} & 1-p_{t,a,s} \\ 0 & 1 \\ 0 & 1 \end{bmatrix}$$

Here, *p* is the probability to resight a marked individual that is present in the study river at a given occasion.

We used a Bayesian implementation of the model. This has the advantage that the latent state of each individual can be estimated, i.e. we know the probability that an individual is present in the study river, is temporary absent, or has permanently emigrated or is dead. In order to illustrate seasonal pattern in the focal parameters, we constrained values of all occasions within a month to be the same, resulting in monthly estimates for each age class and sex and parameter type, respectively. For the Bayesian implementation we closely followed the description as in Kéry and

Schaub (2012). For each parameter, we specified vague priors (uniform between 0 and 1). MCMC settings for the analysis of the data in the multistate model were: three chains of 500'000 iterations, each with a burn-in of 200'000 iterations and a thinning of 200 iterations, resulting in parameters being estimated from 3x 1'500 iterations. Because temporal patterns were similar in both periods, we pooled data to improve identifiability of parameters. We used the program WinBUGS 1.4 (Lunn et al. 2000) that was run from R version 3.0.2 (R Development Core Team 2013) via the R2WinBUGS package for model fitting.

We used resight data from the four periods of four successive days to obtain an independent estimate of detection probability, assuming the population to be closed during each of these periods. We fitted a hierarchical occupancy model to estimate age-specific detection probability (Kéry and Schaub 2012) with the following MCMC settings: three chains of 100'000 iterations and a thinning of 50 iterations after a burn-in of 50'000 iterations. Age-specific detection probabilities were estimated separately for all four periods, using uniform priors.

Results

We used mark-resight data of white-throated dippers to describe and quantify movement patterns between breeding seasons within a river and temporary emigration from this river. Sixty-nine per cent of ringed offspring could be observed at least once (78 of 101 in 2011 and 43 of 74 in 2012). Out of these, 8 females and 13 males recruited into their natal river. A total of 16 juveniles immigrated into the study river, 5 of which eventually became a breeder. In addition, data was collected on 37 adults in the 2011/12 period and 35 in the 2012/13 period.

Fine-scale movement patterns

Juveniles had a higher probability than adults (82.6% vs. 74.7%; difference on logit scale = 0.51 ± 0.14, $z=3.85$, $p<0.001$) of being observed at different locations during two successive surveys. This probability was lowest at the upper and lower end of the river (predicted as 68 and 56% in juveniles and adults, respectively) and highest at a distance of 2km from the ends (86 and 78% in juveniles and adults, respectively; linear term: 1.0 ± 0.3, $z=3.77$, $p<0.001$, quadratic term: $-2.6 \cdot 10^{-4} \pm 7.6 \cdot 10^{-5}$, $z=-3.4$, $p<0.001$). There was no effect of sex (0.20 ± 0.13, $z=1.50$, $p=0.13$). Similarly, when an individual had changed its location, the distance between the two sites was longer in juveniles than in adults (0.22 vs. 0.16km; difference on logarithmic scale = 0.33 ± 0.07, $t=4.62$, $p<0.001$). Covered distances were longest for individuals at the beginning or end of the river (0.34 vs. 0.24km for juveniles and adults, respectively) and lowest in the middle of the river (0.05 vs. 0.04km respectively). Again, there was no effect of sex (0.14 ± 0.10, $t=1.42$, $p=0.16$).

Juveniles were statistically less strongly tied to their natal site than adults to their previous breeding site (Fig. 1a). Juveniles, in particular those born in 2012, had both a higher probability of being located away from this site (difference on logit scale = 1.31 ± 0.29, $z=4.57$, $p<0.001$ for juveniles born in 2011 and 3.34 ± 0.66, $z=5.06$, $p<0.001$ for juveniles born in 2012) and the distance between them and their natal site was on average larger (difference on logarithmic scale = 0.87 ± 0.05, $t=15.93$, $p<0.001$ for juveniles born in 2011 and 1.71 ± 0.07, $t=22.89$, $p<0.001$ for juveniles born in 2012). This pattern was stronger in males than in females (0.48 ±0.28, $z=1.75$, $p=0.08$ and 0.14 ± 0.05, $t=2.80$, $p=0.005$, respectively). Adult individuals were typically observed in comparably close proximity to their former breeding site throughout the year, with the distance being largest during September and October (Fig. 1a). Juveniles, on the other hand, remained within the proximity of their natal site only for a short time. Starting in late May, the distance from the natal site increased considerably in a few weeks, and oscillated at approximately 1.5km afterwards (Fig. 1a).

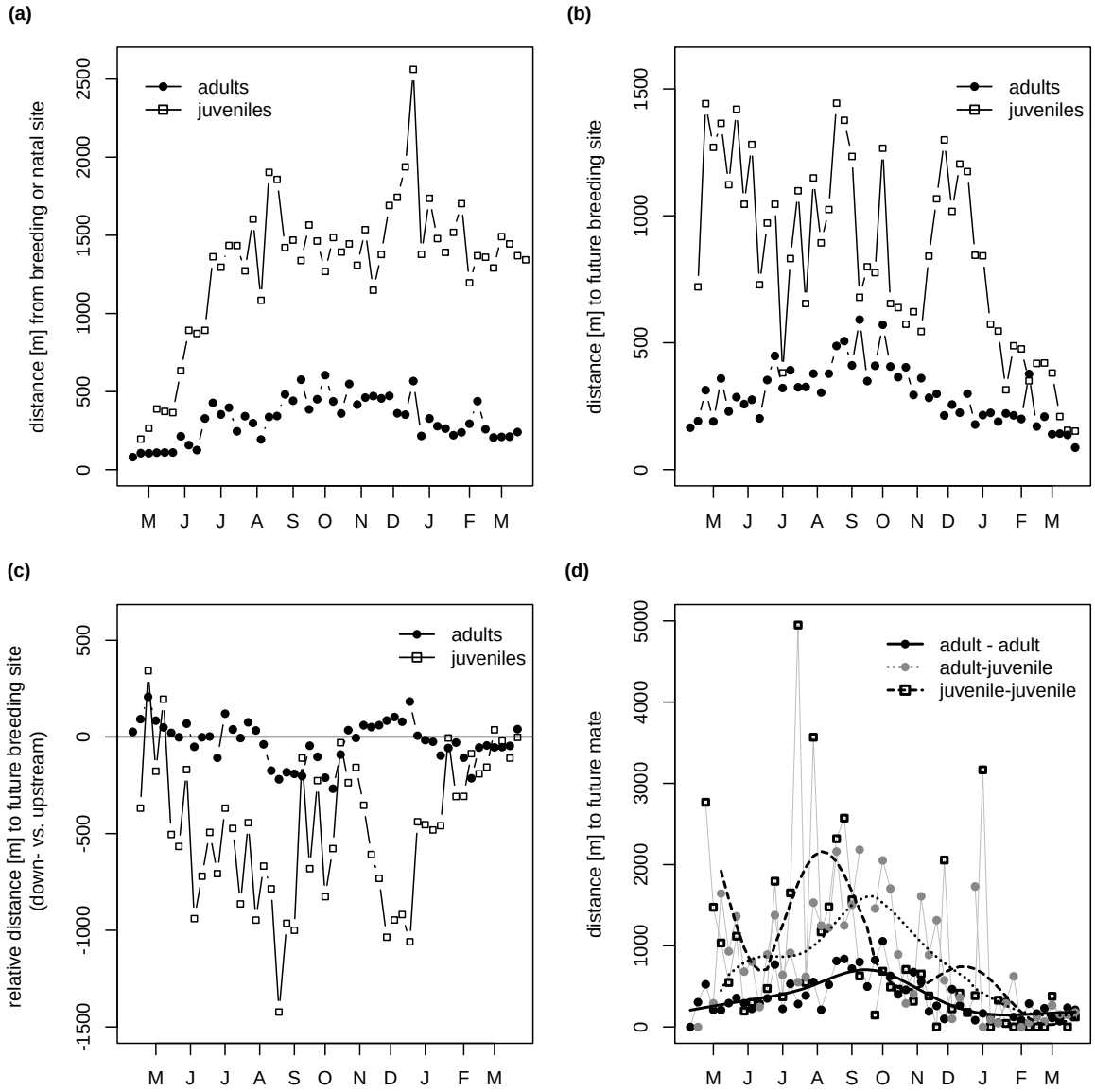

Figure 1: Movement patterns of white-throated dippers within a single river between breeding seasons (May to March of the following year). (a) Mean distance from the natal (juveniles) or breeding site (adults), respectively. (b) Mean absolute distance towards the future breeding site. (c) Mean relative distance to the future breeding site. Individuals observed upstream of this site have a positive distance, those observed downstream a negative value. (d) Mean distance to the future mate. Pairs of two adults are indicated as black circles, pairs of one adult and one juvenile (i.e. a first-year breeder) as grey circles, and pairs of two juveniles as open squares. Solid (adult-adult), dotted (adult-juvenile) and dashed (juvenile-juvenile) curves are smoothed prediction for pairwise distances as from a generalized additive model.

Similarly, average distance to the future breeding site was below 500m throughout the year in adults (n=31, 13 of which were breeding in 2012 and 2013). From October on, they came continuously closer to their future breeding site (Fig. 1b). On average, adults had no preference of staying up- or downstream of their future breeding site (Fig. 1c). Juveniles, which recruited in the following breeding season (n=26), were typically more than 500m away from their future breeding site until January (Fig. 1b). From February onwards, they got closer to their new territory. Interestingly and contrary to adults, juveniles (i.e. new recruits) were on average

observed downstream of their future breeding site (Fig. 1c). Again, both the probability of being observed away from the future breeding site and the distance to it were higher in juveniles than in adults (86.9% vs. 78.0%, difference on logit scale =1.26 ± 0.31, z=4.10, p<0.001; and 872m vs. 368m, difference on logarithmic scale = 0.75 ± 0.10, t=7.71, p<0.001, respectively) and higher in males than in females (86.9% vs. 75.4%, 0.74 ± 0.28, z=2.67, p=0.008; and 596m vs. 473m, 0.18 ± 0.09, t=1.97, p=0.05, respectively).

Finally, we analysed the distance between two future mates (Fig. 1d). If both mates were adult, i.e. if both were already breeding in the previous season (n=20 pairs), they were typically not far apart from each other. Especially from December onwards, they were constantly in rather close proximity. If both mates were juveniles (i.e. new recruits, n=10 pairs), mates were on average separated more than adult-adult pairs with considerable variation. However, with few exceptions, patterns seemed to resemble dynamics of adult-adult pairs already from October onwards. Pairs of one adult and one juvenile individual (n=7) seemed to be spatially distant for a longer time before they eventually approached each other.

Survival and temporary emigration

Using multistate mark-recapture (or: mark-resight in our case) models, mean detection probability (±sd) was estimated as 81.3 ± 10.6% for adults and 75.1 ± 13.3% for juveniles (Fig. S1). Based on the additional hierarchical occupancy model, detection probability was estimated to range between 79 and 90% for adults (mean ± sd of the four periods: 85.6 ± 5.6%) and between 68 and 75% for juveniles (72.2 ± 5.9%).

Apparent (local) survival per month was similarly high for adult females and males (mean of monthly survival rates 98.1 ± 1.7% vs. 97.4 ± 3.5%). While survival was estimated to be nearly constant throughout the year, it was reduced with the beginning of the breeding season in March (Fig. 2a). Annual adult survival, as from the product of monthly estimates, was 72.9% (95% credible interval CRI: 60.9 - 84.2%) in females and 79.5% (95% CRI: 70.6 - 86.9%) in males. Apparent juvenile survival per month between May and March of the following year (females: 94.7 ± 5.0%, males: 94.8 ± 4.4%) was slightly lower than adult survival ($Pr|\phi_{juv}>\phi_{ad}|=0.25$), especially in the first few months post-fledging (see Fig. 2a). From late autumn (October/November) onwards, estimates started converging with adult survival probabilities (Fig. 2a). Juvenile survival between May and March, i.e. excluding early mortality before and shortly after leaving the nest, was calculated as 43.1% (95% CRI: 30.2 - 55.9%) and 50.7% (95% CRI: 41.0 - 60.2%) , for females and males respectively.

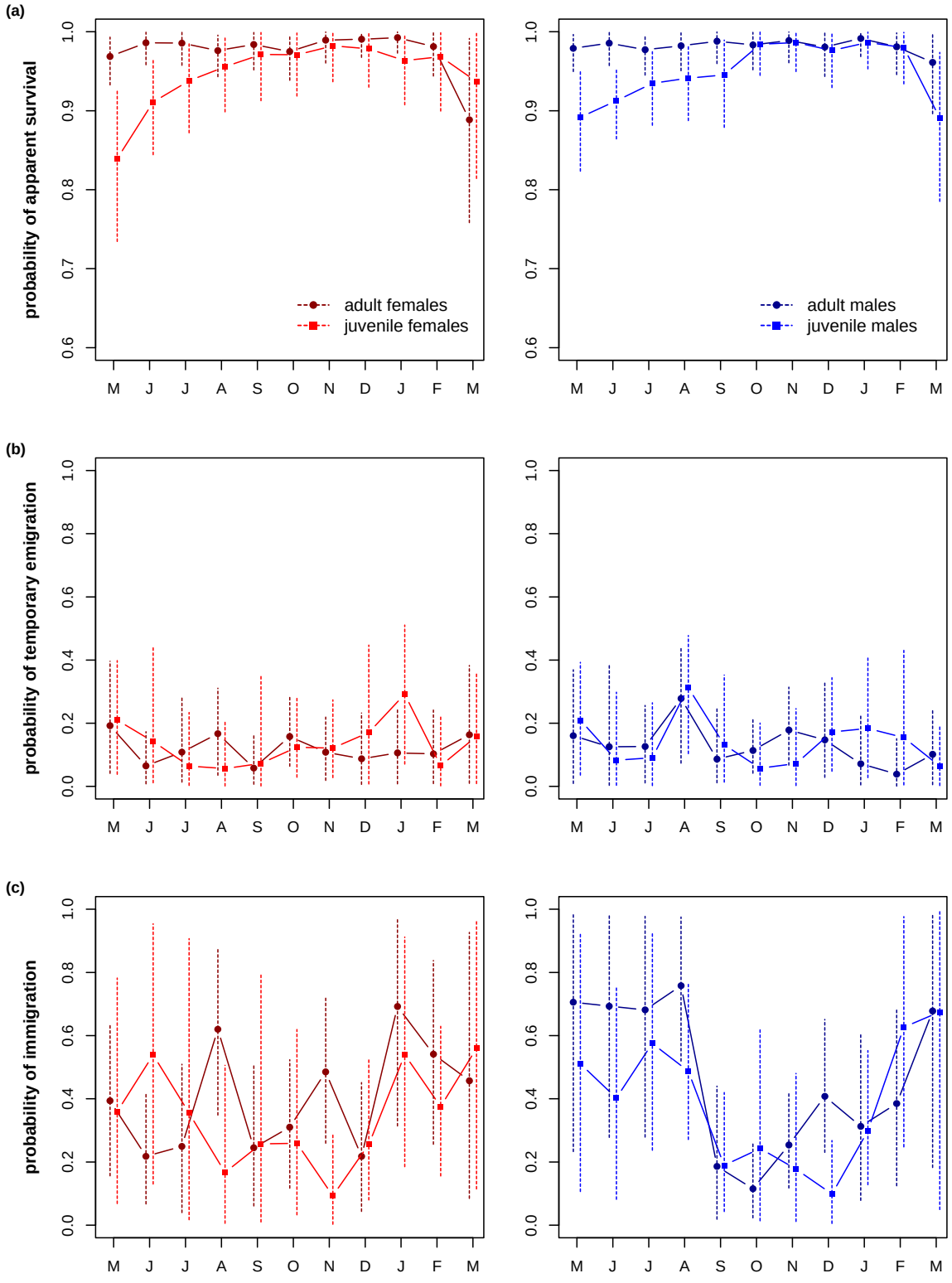

Figure 2: Probabilities of apparent survival (a), temporary emigration (b) and immigration (c). Monthly estimates (May to March of the following year; mean and 95% credible interval) are shown separately for females (left column) and males (right column), and for adults (circles, dark colours) and juveniles (squares, light colours).

Averaged over the year, estimates of temporary emigration (Fig. 2b) did not differ between adults and juveniles (mean of monthly probabilities: 12.5 ± 8.8% vs. 13.7 ± 11.3%) nor between females and males (12.7 ± 9.8% vs. 13.4 ± 10.5%). A closer look at the estimates for juveniles shows that temporary emigration was more likely (though not significantly) when becoming independent (in May) and in winter (December / January), when dippers start becoming territorial. In addition, juvenile males showed high temporary emigration in August. Immigration probability (Fig. 2c) showed temporal patterns, in particular in males. They were less likely to return from temporary emigration between September and December. Afterwards their immigration probability increased markedly. While adult females did not show seasonal patterns, juvenile females showed similar patterns as males.

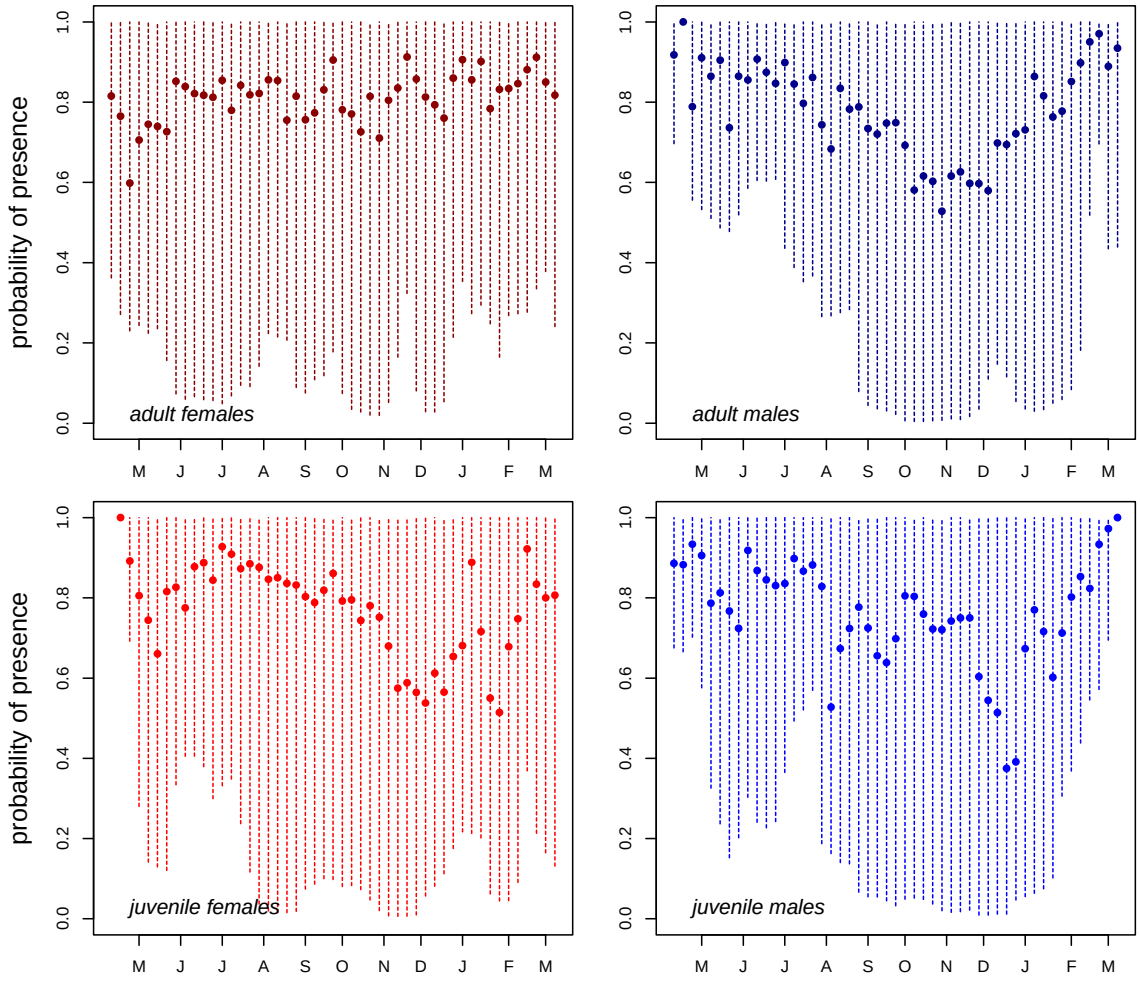

Figure 3: Mean probability of being present in the study river (estimates per occasion), separately for females (red, left) and males (blue, right), and for adults (dark colours, top) and juveniles (light colours, bottom). Vertical bars indicate the 95% confidence intervals of individual means. Large confidence intervals reflect individual variability in presence-absence patterns rather than uncertainty in the estimates. Only individuals that were present during the last three occasions (minimum probability of presence of 0.4) were used for this analysis (19 adult females, 22 adult males, 15 juvenile females, 13 juvenile males). Data of both years combined.

The latent state variable in the multistate model allows for calculating the probability of an individual being present in the study river at a particular occasion. We derived this probability only for individuals, which were still present at the beginning of the next breeding season (Fig. 3). Adult males were less like to be present between September and January but showed high presence with the onset of the breeding season. In adult females, temporal patterns were less obvious, with the entire adult female population being present at some occasions. Like adult males, a considerable proportion of both female and male juveniles was absent between birth and the first breeding season, in particular between September and January. Patterns of presence and absence, however, differed strongly between individuals, also for individuals with the same sex and age. Some individuals were almost always present while others were considered being temporary absent for periods of different lengths (see Fig. 4).

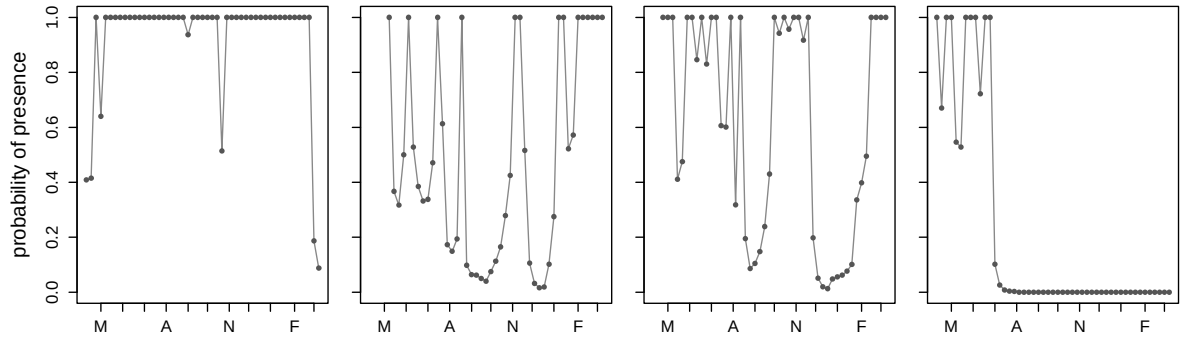

Figure 4: Probability of being present in the study river for four different individuals. While the first individual stayed in the river almost throughout the year (adult female), two other individuals showed patterns of successive temporary emigrations (two juvenile males) and one individual either died or emigrated permanently in July (adult male). Labels on the x-axis indicate months (May, August, November 2011 and February 2012).

Discussion

Dispersal is one of the most important life-history traits and therefore receives on-going attention. While most studies focus on dispersal distance and rate or try to identify factors that influence emigration and settlement, data on the spatiotemporal process of dispersal itself are still scarce. Here, we used mark-resight data of white-throated dippers to obtain unique insights into the movement behaviour between breeding seasons that constitutes dispersal.

In juveniles, the distance from the natal site increased rapidly after becoming independent. We suggest that this is the first step (emigration from the place of birth) of a three-phased dispersal process (sensu Clobert et al. 2012). Juveniles that hatched before the end of April (i.e. mainly offspring from first broods) started this process on average at the end of May, at an approximate age of 50 days. Juveniles that hatched later (i.e. mainly offspring from replacement and second broods) did so in the second half of June. Especially against the background that on average this distance remained more or less constant afterwards, it is this movement that is expected to reduce kin competition and make inbreeding less likely. Both avoidance of kin competition and inbreeding are considered as major drivers for the evolution of dispersal behaviour (see Clobert et al. 2012). Notably, the observed mean distance from the natal site (appr. 1.5km, Fig. 1a) resembles the mean natal dispersal distance of philopatric individuals in this river (1.85km in females, 1.36km in males, n=54 and 73, respectively, data from 1996-2013).

During the first months after fledging, estimates of apparent survival were comparably lower than later in life. In birds, juvenile mortality is typically highest during fledging, and continues to be high until after they become independent from parental care (see Kershner et al. 2004 and references therein). However, apparent survival underestimates true survival due to permanent emigration of dispersing individuals (Lebreton et al. 1992). Indeed, our long-term data show that juveniles start leaving their natal river already in June (with two exceptions in May). Thus, juveniles do not only move away from their natal site within the natal population, but also start emigrating to different populations, either permanently or temporarily. Those that emigrated temporarily, however, had a high probability of returning to their natal population shortly afterwards (Fig. 2c). As opposed to juveniles, adult dippers did not show this spatiotemporal movement pattern, reflecting the fact that breeding dispersal is typically much less pronounced than natal dispersal (Greenwood and Harvey 1982, Paradis et al. 1998). Specifically, breeding dispersal in the study river is on average only over 0.15km in females and 0.14km males (n=119 and 115 dispersal events of females and males, respectively, between 1996 and 2013, see also Fig. 1a).

We consider the time period between emigration from the natal territory and approaching the future breeding site (i.e. settlement; see below) as the transient phase of dispersal. Despite juveniles being considerably more mobile than adult individuals (accounting for the position within the river, relative to its upper and lower end), this did not result in a further increase in the average distance from the natal site in the transient phase. Interestingly, juveniles spent this transient phase on average downstream of their future territory. Environmental conditions in rivers are typically more benign further downstream due to more constant water levels as well as higher food availability and water temperature.

In addition, presence-absence data revealed signs of temporary emigration during this period in juveniles, especially during autumn and early winter, but with substantial inter-individual variability. We suggest that both the higher mobility and temporary emigration in juveniles are a form of exploratory behaviour, allowing them to find open territories and potential mates. In a study on two Australasian treecreeper species (Climacteridae), spatial and temporal aspects of search and exploration behaviour were likewise found to vary strongly among individuals (Doerr and Doerr 2005). Indeed, exploratory behaviour is considered to be a major component of dispersal (Reed et al. 1999, Conradt et al. 2001, Dingemanse et al. 2003, Doerr and Doerr 2005, Cote et al. 2011). For example, in semi-natural enclosures, common voles that entered but did not settle into a new population, continued dispersal, typically on the same day (Hahne et al. 2011). Field studies on two butterfly species suggest that repeated short excursions outside the home patch allow the exploration of the surrounding environment (Conradt et al. 2000, Conradt et al. 2001). These systematic search strategies enable individuals to (repeatedly) return to their familiar home patch, to increase familiarity over a larger area and possibly also settlement success of dispersers (Conradt et al. 2001). Thus, exploratory behaviour allows informed dispersal and is expected to occur frequently, especially in mobile species like birds due to its potential advantages (Reed et al. 1999). In our case, it might enable juveniles to explore adjacent rivers, even if they eventually return and settle in their natal river. Exploratory behaviour has also been described as part of a personality syndrome, which, in turn, has been linked to dispersal behaviour (Dingemanse et al. 2003, Cote et al. 2011, Korsten et al. 2013). Thus, linking mobility and the propensity for temporary emigration with dispersal behaviour is worth further investigation, not only in this species but also in other species with similar data (like telemetry data) and larger sample sizes.

Mark-resight data from a single population lack information on the whereabouts of individuals, which have emigrated either temporarily or permanently. That means we do not know whether juveniles explored several populations or only one population, once or repeatedly, or whether

individuals explored their future breeding site or population already earlier and thus possibly compared it to other potential sites or populations. On-going studies on other species using radio or satellite telemetry techniques will generate large data set that can hopefully answer these questions.

From January onwards juveniles started to approach their future breeding site. Note however that while individuals approached their future breeding site, the *average* distance from the natal site did not change, with the latter being equal to the average dispersal distance (see above). Further analysis of movement paths can provide additional data on the number of open and occupied territories an individual visits, which would help to improve our understanding of how settlement decisions are made.

While the process of mate choice has been studied in captivity in a wide range of species (Milinski and Bakker 1990, Thünken et al. 2007), its quantification in free-ranging animals remains problematic (but see Qvarnström et al. 2000, e.g. Postma et al. 2006, Szulkin et al. 2013). Here, we quantified the distance between two future mates, which provides an insight into temporal aspects of mate choice. Provided both were present in the local population, pairs of adult individuals typically were in close proximity throughout the year. On the other hand, the distance between two juveniles (i.e. two new recruiting individuals) was highly variable across summer and autumn. However, from October onwards, they started to approach each other, and patterns started to resemble those for adult individuals. This suggests that mate choice precedes the process of settlement. Although little data is available, pair bonds between an adult and a juvenile appear to form later than those between two juveniles. As adults typically mate with their previous partner, in those cases when last year's partner is not present in the population, either because it is dead or it has (temporarily) emigrated, adults might wait whether he or she might return from temporary emigration before engaging in a new pair bond with an unpaired juvenile. On the whole, these data show that movement data can give new insights into the process of mate choice in the wild, and that they might facilitate the formulation of hypotheses about the role mate availability has in shaping patterns of mate choice (Pärt 1996, Keller and Arcese 1998, Szulkin et al. 2009, Szulkin et al. 2013).

In summary, we conclude that explorative behaviour, including temporary emigration, should be considered as an important component of dispersal behaviour, also in the management of endangered species living in fragmented environments. It allows juveniles to become not only familiar with the natal but also with adjacent populations. Importantly, we suggest that it is an important component of the movement behaviour of all individuals, including those that eventually breed in their natal population. Thereby, this raises question about the cost of dispersal,

which in terms of the amount of ground covered during transience might be similar for dispersing and non-dispersing individuals. However, as of yet the fitness consequences of variation in exploratory behaviour are unknown and deserve future research.

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Appendix

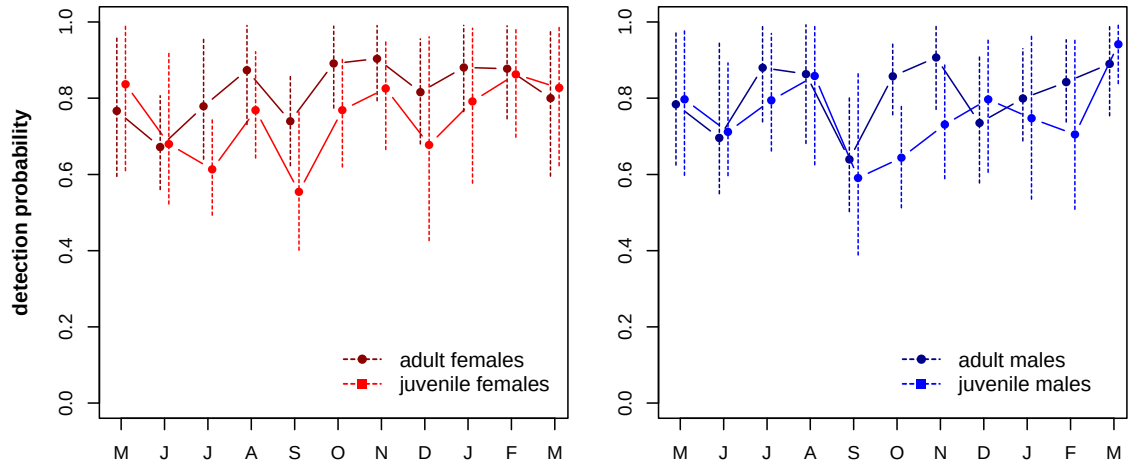

Figure S1: Estimates of detection probability from a multistate mark-recapture model. Monthly estimates (May to March of the following year; mean and 95% credible interval) are shown separately for females (left) and males (right), and for adults (circles, dark colours) and juveniles (squares, light colours).

Chapter 3

How dispersal shapes genetic variation and patterns of inbreeding in a bird species inhabiting a naturally fragmented environment

Philipp J. J. Becker, Johann Hegelbach, Lukas F. Keller, Erik Postma

Abstract

Dispersal is a key life-history trait that is of relevance to various ecological and evolutionary processes. As a mediator of gene flow, it shapes spatial patterns of genetic variation and relatedness. Thereby, dispersal can be an effective means of inbreeding avoidance, especially in small and isolated populations. Here we use both mark-resight and genetic data for white-throated dippers (*Cinclus cinclus*), a bird species living exclusively along rivers, to quantify patterns of dispersal and genetic variation within and across their naturally fragmented environment, and relate these to patterns of inbreeding. Mark-resight data revealed female-biased dispersal, with a high proportion of birds dispersing over short distances and breeding in their natal river. Nevertheless, additional ring recovery data show that despite the relatively large size of our study area, dispersal kernels based on within-study area movements substantially underestimate the frequency of long-distance dispersal events. In line with this, genetic data revealed only weak genetic differentiation between rivers, even on a large spatial scale, but substantial levels of genetic structure on the small (within-river) scale. Furthermore, we find that philopatric individuals have high probabilities of inbreeding, in particular if they are female and disperse over very short distances within their natal population. We conclude that in this species, inbreeding is common because of the linear and fragmented nature of their breeding habitat, typically sustaining only small population sizes, combined with limited amounts of dispersal. Still however, our data suggest that female-biased dispersal contributes substantially to reducing the risk of inbreeding. Furthermore, our findings highlight the fact that weak genetic differentiation among populations does not exclude the frequent occurrence of inbreeding within populations, in particular in small populations of species living in fragmented habitats. Importantly, this includes many species of conservation concern.

Keywords: female-biased dispersal · genetic variation · spatial autocorrelation · inbreeding · white-throated dipper

Introduction

Dispersal is the movement of individuals between birth and first breeding (natal dispersal), or between successive breeding events (breeding dispersal)(Greenwood and Harvey 1982). In a spatially structured environment, these movements can be both within and between different spatial units. Thereby dispersal shapes population dynamics and structure. When dispersing individuals pass their genes to the next generation, dispersal results in the movement of genes (i.e. gene flow) and thereby affects spatial patterns of genetic variation. Thereby, dispersal is relevant on various ecological and evolutionary levels (Clobert et al. 2012).

The movement of individuals and genes among habitat patches may be restricted due to the restricted dispersal abilities of the individuals inhabiting these patches, and due to ecological barriers in between patches of suitable habitat (Ahlroth et al. 2010, Kekkonen et al. 2011), resulting in reduced effective population sizes and thereby increased amounts of genetic drift. This will result in an increase in genetic diversity among patches (i.e. population differentiation), and a loss of genetic diversity within habitat patches due to genetic drift (Gillespie 2004).

Inbreeding, i.e. the reproduction among relatives due to either non-random mating or genetic drift, is a common phenomenon in small and isolated populations (Lande 1988, Keller 1998, Reid et al. 2014), and numerous studies have shown it to have negative consequences (i.e. inbreeding depression), both in captive and wild populations (Charlesworth and Charlesworth 1987, Keller and Waller 2002). Depending on the costs of inbreeding and its avoidance, inbreeding avoidance mechanisms could be expected to evolve (Kokko and Ots 2006, Lawson Handley and Perrin 2007). Although some studies find evidence for a preference for genetically dissimilar social or extra-pair partners (see also Foerster et al. 2006, e.g. Arct et al. 2010), a number of studies testing for active inbreeding avoidance do not find any deviation from random mating within the local population (Keller and Arcese 1998, Wheelwright and Mauck 1998, Hansson et al. 2006, Szulkin et al. 2009, Billing et al. 2012, Szulkin et al. 2013). Instead, dispersal has been suggested to have evolved as a mechanism to avoid inbreeding that does not require the active avoidance of relatives (Hamilton and May 1977, Gandon and Michalakis 2001, Guillaume and Perrin 2006). In particular, a sex-bias in dispersal might already contribute substantially to inbreeding avoidance, making other mechanisms of kin discrimination less important (Pärt 1996, Lebigre et al. 2010). However, the number of empirical studies investigating the effect of dispersal in reducing the probability of inbreeding is still low (Schiegg et al. 2006, Van de Casteele and Matthysen 2006, Szulkin and Sheldon 2008, Lebigre et al. 2010).

Understanding the effects of individual dispersal behaviour on the occurrence of inbreeding requires a comprehensive picture of dispersal and its resulting gene flow. Both can be described and quantified with a number of different methods, using either observational data (like mark-recapture or mark-resight data) or genetic data (Lawson Handley and Perrin 2007). For example, dispersal patterns can be inferred from field observations by marking individuals at their natal site and resighting them later in life. Data from such individual-based long-term studies can be used to summarize all dispersal movements within the study area (e.g. Greenwood 1980, Koenig et al. 2000, Dingemanse et al. 2003, Hegelbach 2008, Szulkin and Sheldon 2008). This provides us with the density distribution of dispersal distances, i.e. the dispersal kernel, which can also be used to infer sex-biased dispersal patterns. If the species' distribution is spatially structured and the study area is large enough to cover multiple fragments, dispersal between these fragments can be quantified as well. However, dispersal beyond the (often arbitrary) borders of the study area will usually remain undetected (Van Noordwijk 1984, Koenig et al. 2000). Thus, very often, dispersal kernels will not reflect true dispersal patterns, as they will miss the tail end of the distribution, in particular in mobile species. Tracking methods, including GPS devices and radio telemetry techniques (Aebischer et al. 2010, Griesser et al. 2014, Kissling et al. 2014), as well as the use of large-scale ring-recovery data in birds (Thomson et al. 2003, Kekkonen et al. 2011) have the potential to reduce this bias, at least in some species.

Assuming that dispersal patterns match patterns of gene flow, genetic data offer an alternative, indirect, way of quantifying dispersal (Prugnolle and de Meeus 2002, Lawson Handley and Perrin 2007, Broquet and Petit 2009). To this end, Wright's F-statistics and a number of related measures of genetic differentiation and distance have been derived and extensively discussed (e.g. Jost 2008, Meirmans and Hedrick 2011, Whitlock 2011), providing estimates of the amount of genetic variation within and/or among populations. Some of these measures can sometimes be used to infer levels of gene flow (Whitlock and McCauley 1999). In addition to these population-level estimates of gene flow, individual-based approaches can describe spatial genetic structure within populations (see Rousset 2000, Watts et al. 2007). For example, spatial autocorrelation analysis (Smouse and Peakall 1999) can visualize how pairwise genetic similarity changes with spatial separation. If gene flow occurs mainly over short distances, two individuals in spatial proximity are expected to be genetically more similar than two individuals from random locations, and spatial autocorrelation will thus be positive over short distances.

Here we use data from an individual-based long-term study of white-throated dippers (*Cinclus cinclus*) to investigate the effects of dispersal on spatial genetic structure and patterns of inbreeding. This bird species lives exclusively along rivers and hence inhabits a naturally fragmented environment. Within a single river, their distribution is typically continuous and linear and depends on the length of the suitable river habitat. Population size, i.e. the number of breeding individuals within a river or a stretch of suitable river habitat, is typically small and ranges between a single pair and a few tens of pairs. Thereby dippers make an excellent system to study dispersal and gene flow in a naturally fragmented environment and its consequences for patterns of inbreeding.

We first describe dispersal behaviour both within and between populations and test for sex-specific differences, using observational data from our long-term study, complemented with ring recovery data from outside our study area. Subsequently, we analyse the consequences of dispersal behaviour on (1) the genetic structure and (2) patterns of inbreeding at different spatial scales. Using a combination of observational and genetic data we show that dispersal in spatially fragmented environments can generate both genetic structure over a small spatial scale and high levels of inbreeding. At the same time, there may still be considerable gene flow among populations, leading to weak differentiation at the population level. This gene flow is female-biased and seems to be maintained by a higher risk of inbreeding for females than males when staying near their place of birth.

Material and Methods

Study system

The white-throated dipper is a medium-sized passerine (in our study population males are 62.5 ± 3.6g and females 53.7 ± 4.1g; mean ± s.d.) that is widely distributed across Europe. Dippers live along rivers and mainly feed on aquatic invertebrates. After an incubation time of 16-17 days, offspring of the first brood (brood size at ringing: 4.4 ± 1.1 nestlings) hatch between the middle of March and the beginning of May. About 35% of all offspring are from second broods (Hegelbach 2013) with 3.7 ± 1.1 nestlings hatching between the end of April and the beginning of June. Both parents provide food to the offspring, which fledge at the age of 21-24 days (Schoop 1997).

Since 1987, dippers of the subspecies *C. c. aquaticus* have been monitored intensively at up to eleven rivers, spanning an area of approximately 20 x 20km in the proximity of Zurich, Switzerland (8°23'E / 47°25'N to 8°40'E / 47°10'N, see Fig. 1 in Chapter 1). In three rivers (Küsnacht [suitable river habitat including smaller side rivers: 8.0km], Wehrenbach [7.0km] and Sihl [25.5km of suitable habitat are monitored]) virtually all parental individuals are known (between 1996 and 2013 only 0.1% and 0.5% of ringed nestlings have an unknown mother or father, respectively) and less than 1% of all broods was inaccessible. In two further rivers (Reppisch and Jonen [20.5 and 17.km of suitable river habitat, respectively]), monitoring started later (1997 and 2001, respectively) and some occupied territories may have been missed. The remaining rivers harbour only very small populations (<5 pairs) and have not been monitored continuously.

At an age of approximately 9-14 days, we colour-ringed and measured all offspring and since 2001 we take a blood sample by puncturing the tarsal vein in some rivers. Adults without rings (i.e. immigrants) were captured using mist nets, usually before the breeding season, but at the latest before their offspring were ringed. Like nestlings, they were ringed, measured and a blood sample was taken (since 2001).

To estimate genetic differentiation on a larger spatial scale we took additional blood samples in two populations in the cantons of Ticino (n=32; 8°44'E, 46°13'N, 125km south of Zurich and across the Alps) and Jura (n=30; 7°22'E, 47°21N, 90km east of Zurich) during the early breeding season in 2013 (27 February to 15 April).

Estimation of inbreeding coefficients and the probability of inbreeding

Behavioural observations during the mating and breeding season allowed determining parentage of each brood. Because dippers have a very low rate of extra-pair paternity (2% according to Øigarden et al. 2010; less than 1% according to our own unpublished data), we reconstructed the pedigree based on behavioural observations. We calculated Wright's inbreeding coefficient (*f*) for all individuals since 1987 using the software Pedigree Viewer (available at http://www.personal.une.edu.au/~bkinghor/pedigree.htm). Because founders and immigrants by definition have unknown parents and are therefore assigned an (uninformative) inbreeding coefficient of zero, all analyses are restricted to individuals that have hatched in the study area. We also give inbreeding coefficients from individuals with all four grandparents known (Keller 1998). We excluded individuals that hatched in 1995 or earlier because monitoring was still incomplete in the first years of the study and levels of inbreeding might thus be underestimated.

Subsequently, we estimated for both sexes the probability of pairing with a related mate (defined as a kinship coefficient of ≥0.03125, see Szulkin and Sheldon 2008) and compared this probability among philopatric individuals (breeding in their natal river), dispersers (defined as dispersing between rivers within the study area), and immigrants (defined as dispersing into our study area from outside). In addition, we tested whether the probability of the mate being related (modelled as a binary trait with individuals being either related or not) changed with natal dispersal distance. For this analysis only philopatric individuals in two rivers (Sihl with a length of 25.5km and Küsnacht with a maximum length of 6.5km) were considered, because population size is always above 10 pairs in these rivers, allowing for a reasonable sample size.

Dispersal kernels

We measured natal dispersal distance as the straight-line distance between the place of birth and the place of first breeding. The resulting dispersal kernel is based on all known dispersal movements within the study area since 1996 and hence includes both within- and between river dispersal events. We subsequently classified individuals according to their dispersal behaviour into philopatric individuals (i.e. the river of birth is the river of breeding), dispersers (i.e. dispersal occurred between different rivers of the study area) and immigrants (i.e. individuals of unknown origin), using data of the three best monitored rivers (Küsnacht, Sihl and Wehrenbach). To obtain an estimate of the dispersal distance of immigrants, we used ring recovery data from the Swiss Ornithological Institute, which provides us with an approximate estimate of dispersal distance of non-philopatric individuals. It should be mentioned that these individuals might not have been

recovered at their place of reproduction, i.e. distances might deviate from the dispersal distance. Subsequently, we quantified breeding dispersal as the distance between breeding sites in consecutive years.

Measuring connectivity

Based on river-specific mark-resight data, we used a measure of connectivity that allowed us to quantify the bidirectional exchange of individuals between different pairs of rivers (Postma 2005). Under the null hypothesis of random dispersal recruiting individuals are randomly distributed across all i rivers, including the river of birth, proportionately to the number of individuals breeding in each river. Then the expected number of individuals born in river x and breeding in river y is:

$$n_{x,y\ (observed)} = \frac{n_{x,T} \cdot n_{T,y}}{\sum_{j=1}^{i} n_{T,j}},$$

with $n_{x,T}$ being the total number of individuals born in x and $n_{T,y}$ being the total number of individuals breeding in y. However, when $n_{x,y}$ is also a function of connectivity between river x and y ($c_{x,y}$) and all other rivers, then

$$n_{x,y\ (observed)} = \frac{n_{x,T} \cdot c_{x,y} \cdot n_{T,y}}{\sum_{j=1}^{i} c_{x,j} n_{T,j}}.$$

Because $c_{x,y}$=1 if x=y and because the denominator is the same for all $n_{x,1..i}$ connectivity between two rivers can be calculated using mark-resight data as

$$c_{x,y} = \frac{n_{x,y} \cdot n_{T,x}}{n_{x,x} \cdot n_{T,y}}.$$

Connectivity is therefore proportionate to the number of individuals that have been recorded as breeding in each river. Note that $c_{x,y}$ can be different from $c_{y,x}$, because the direction of dispersal matters here. If there was no dispersal between two rivers, connectivity will be zero. We calculated connectivities based on mark-resight data from five rivers (Küsnacht, Sihl, Wehrenbach, Reppisch and Jonen), using all available data since 1996. Subsequently, we tested whether connectivities between two rivers in both directions were correlated. Significance of the correlation coefficient was evaluated using matrix permutation tests with 10’000 permutations of rows and columns of one matrix. We then calculated mean connectivities between rivers, separately for females and males. We tested for a correlation between the female and the male connectivities and whether they were different from each other. Again, significance was tested with permutations tests as outlined above.

Microsatellite data

We used 16 polymorphic autosomal microsatellite markers (Cici02, -04, -05, -06, -07, -08, -09, -11, -13, -14, -16, -17, -22, -23, Ase64 and QmAAT31 in Bucher et al. 2009), of which 14 have been specifically developed for the dipper, amplified with two multiplex PCR's (Gene Amp® PCR System 9700, ABI). Fragment analysis was performed on ABI Prism® 3100 and 3730 Avant DNA analysers and allele sizes were scored using GeneMapper v4.1^{TM} software (ABI). One locus (Cici09) was only included for analyses of recent years (2011 to 2013) due to scoring errors in previous years. Using genotypes of offspring that hatched 2012 and 2013 and their parents, we estimated error rates (allelic dropout and false allele rates, the latter also including genotyping errors) with the R package MasterBayes (Hadfield et al. 2006). Analysis was performed with 500'000 iterations of a Markov chain Monte Carlo search with a thinning interval of 50, discarding the first 25% of iterations. After excluding two Z-linked loci and two loci with an allelic dropout rate >5%, error rates for the remaining 16 markers were low (mean of 0.4% allelic dropout and 1.3% false alleles). We tested for deviations from Hardy-Weinberg equilibrium and calculated observed and expected heterozygosity for each river using Arlequin version 3.5 (Excoffier et al. 2005, Excoffier and Lischer 2010). No marker deviated significantly from Hardy-Weinberg equilibrium in any of the studied populations after Bonferroni correction. Observed heterozygosity ranged between 0.74 and 0.81 and expected heterozygosity between 0.75 and 0.79 (Table S1). As an additional measure of genetic variation we calculated the standardized number of alleles per locus (sNa), which is independent of the sample size, using FSTAT version 2.9.3.2 (Goudet 2001). sNa ranged between 7.29 (Küsnacht) and 8.69 (samples from Ticino) (Table S1).

Spatial autocorrelation analysis

Short-distance dispersal creates fine-scale genetic structure that can be detected using genetic data in a spatial autocorrelation framework as developed by Smouse and Peakall (1999). In essence, autocorrelations are based on two matrices, a pairwise geographic and a pairwise genetic distance matrix, where the latter is based on multi-locus multi-allelic genotype data (Smouse and Peakall 1999). Autocorrelation coefficients (r) were calculated for sets of paired observations, which fall within a certain distance interval of equal size. We considered the correlation coefficient r to be significant if the 95% bootstrap interval did not overlap with zero and the 95% confidence interval for the null hypothesis (determined with a permutation test) did not include r. When individuals within a given distance class are genetically more similar than expected by chance, r is positive.

Multivariate global autocorrelation analyses were performed using the software GenAlEx version 6.5 (Peakall and Smouse 2006, 2012). First, we included all adult individuals breeding in five rivers (Küsnacht, Wehrenbach, Sihl, Reppisch, Jonen) between 2012 and 2013. To analyse the effect of sex-biased dispersal on fine-scale genetic structure, we subsequently repeated the analysis by only including female and male individuals, respectively (cf. Athrey et al. 2012, Banks and Peakall 2012). Second, we performed the analysis separately for the Küsnacht and the Sihl river, using a data set of individuals breeding between 2004 and 2013. However, because genetic distance between individuals is expected to increase with time due to the effects of genetic drift, selection, and mutation (see Fig. S1), we excluded comparisons of individuals that did not co-occur in time from the analysis. We did so by assigning them a geographic distance outside of the range that was considered in the analyses.

Genetic differentiation and genetic distance between populations

To describe population-level genetic structure, we used pairwise F_{ST}-values (Weir and Cockerham 1984) and Nei's genetic distance D (Nei 1978), which readily allow for comparison with microsatellite-based estimates from other studies. Pairwise F_{ST}-values were calculated in Arlequin version 3.5 (Excoffier et al. 2005, Excoffier and Lischer 2010) and genetic distances D between populations in SPAGeDi version 1.4 (Hardy and Vekemans 2002). In order to investigate how genetic variation is distributed among rivers, we grouped rivers from the study area in the canton of Zurich, and included samples from the Ticino and the Jura as two further groups. Subsequently we conducted a hierarchical AMOVA in Arlequin version 3.5 (Excoffier et al. 2005, Excoffier and Lischer 2010).

Results

Dispersal behaviour

More than half of all individuals (39% of females, 65% of males) were philopatric (Table 1). Their dispersal distances were female-biased, with 2.9km in females (95% confidence interval [CI]: 0.5-7.9km, n=151) and 1.8km in males (95% CI: 0.0-6.1km, n=221) (Fig. 1). In both females and males, dispersal occurred on average over shorter distances than if dispersal of these individuals was random within the natal river (mean random distance of females: 3.4km, 95% CI of means: 3.1-3.8km, n=1'000 simulations; males: 3.6km, 95% CI: 3.3-3.9km). One hundred recruiting individuals (13% of all females, 7% of all males) were of known origin but had been ringed as nestlings in a different river. Mean dispersal distances were on average 7.0km (95% CI: 2.7-14.4km, n=63) and 5.2km (95% CI: 2.0-14.3km, n=37) for those females and males, respectively (Fig. 1). Again, these distances were shorter than if these individuals had dispersed to a random site in a different river of the study area (females: 8.8km, 95% CI 7.9-9.6km; males: 8.6km, 95% CI: 7.4-9.9km).

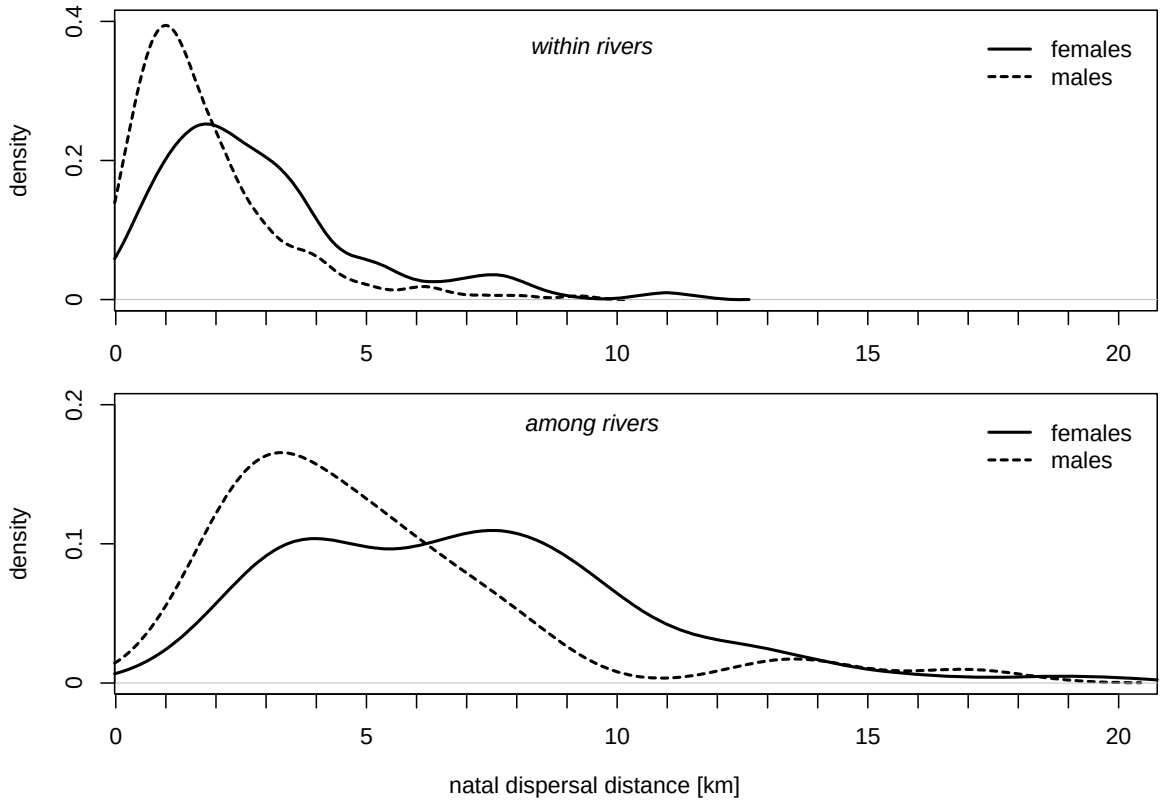

Figure 1: Dispersal kernels of females and males, separately for dispersal within the natal river (philopatric individuals) and dispersal among rivers within the study area (dispersing individuals).

Immigrants contributed substantially to the local breeding population, with 48% of females and 28% of males being of unknown origin (Table 1). Although none of these were ringed, 19 individuals (11 of known sex) which were ringed as nestlings in our study area were recovered by other people at a different river (or its proximity) than the natal river, and reported to the Swiss Ornithological Institute. On average, they were recovered 19.4km (min. 2.9, max. 59.1km) from their location of birth, and reported distances were shorter for males (7.6km, n=4) than for females (27.1km, n=7). Furthermore, one male dispersed over an exceptionally long distance of 1'055km to Gdansk (Poland), where it reproduced with a female from the subspecies *C. c. cinclus* ringed in Sweden, which herself dispersed 828km (Hegelbach and Koch 1994).

Assuming that these distances are a proxy for dispersal distance and that immigrants resemble recovered emigrants in terms of their dispersal distance, we can use these ring recovery data (excluding the Poland bird as an extreme outlier) to indirectly infer the dispersal distance of immigrants. Based on the proportion of individuals being philopatric, dispersers or immigrants and the respective dispersal distances, we estimated the mean population dispersal distance as approximately 9.3km (15.1km in females and 3.6km in males, Table 1). Excluding the estimate for immigrants, the estimate would be substantially lower (3.9km and 2.1km respectively).

Table 1: Proportions of philopatric (dispersal within the natal river), dispersing (dispersal among rivers within the study area) and immigrating individuals (with unknown origin). The dispersal distances of philopatric and dispersing individuals are based on mark-resight data within the study area. The distances for immigrating individuals are estimated from a small number of individuals (7 females and 4 males) that was ringed as nestling in the study area, recorded at a different river and reported to the Swiss Ornithological Institute.

	females		males	
	proportion	distance [km]	proportion	distance [km]
philopatric individuals	39.2%	2.88	65.5%	1.78
dispersing individuals	12.6%	6.97	6.7%	5.20
immigrating individuals	48.2%	≈27.1	27.8%	≈7.6
	100.0%	≈15.1	100.0%	≈3.6

Breeding dispersal occurred over much shorter distances and typically within the same river. Distances between breeding sites in successive breeding seasons were 0.17km (95% CI: 0-1.5km, n=487 movements) in females and 0.20km (95% CI: 0-1.1km, n=454 movements) in males. Only two females (out of 485) and five males (out of 401) were recorded as breeding in two different rivers. Importantly, successive breeding sites were on average (±sd) at approximately equal distances from the natal site than the first breeding site (-0.06km ± 0.64km).

Fine-scale spatial genetic structure

Using pairwise comparisons of breeding individuals from the entire study area (n=252, Fig. 2a), spatial genetic autocorrelation was positive up to 5.8km. Spatial structure was significantly positive for the first three distance classes ($r_{0\text{-}1.5}$=0.038, $r_{1.5\text{-}3.0}$=0.037, $r_{3.0\text{-}4.5}$=0.015, all p<0.001) and zero or slightly negative for larger distances (r=0 at 5.8km). Subsequently, we analysed genetic structure separately for females (n=135, Fig. 2b) and males (n=117, Fig. 2b). Positive spatial autocorrelation was stronger in males than in females over short distances ($r_{0\text{-}1.5}$=0.057 and $r_{1.5\text{-}3.0}$=0.045 vs. $r_{0\text{-}1.5}$=0.033 and $r_{1.5\text{-}3.0}$=0.029, all p<0.001). Thereafter positive structure decreased more strongly in males ($r_{3.0\text{-}4.5}$=0.009, p=0.05) than in females ($r_{3.0\text{-}4.5}$=0.020, p<0.001), resulting in a smaller x-intercept for males than for females (r=0 at 6.3km for females and 5.5km for males, respectively).

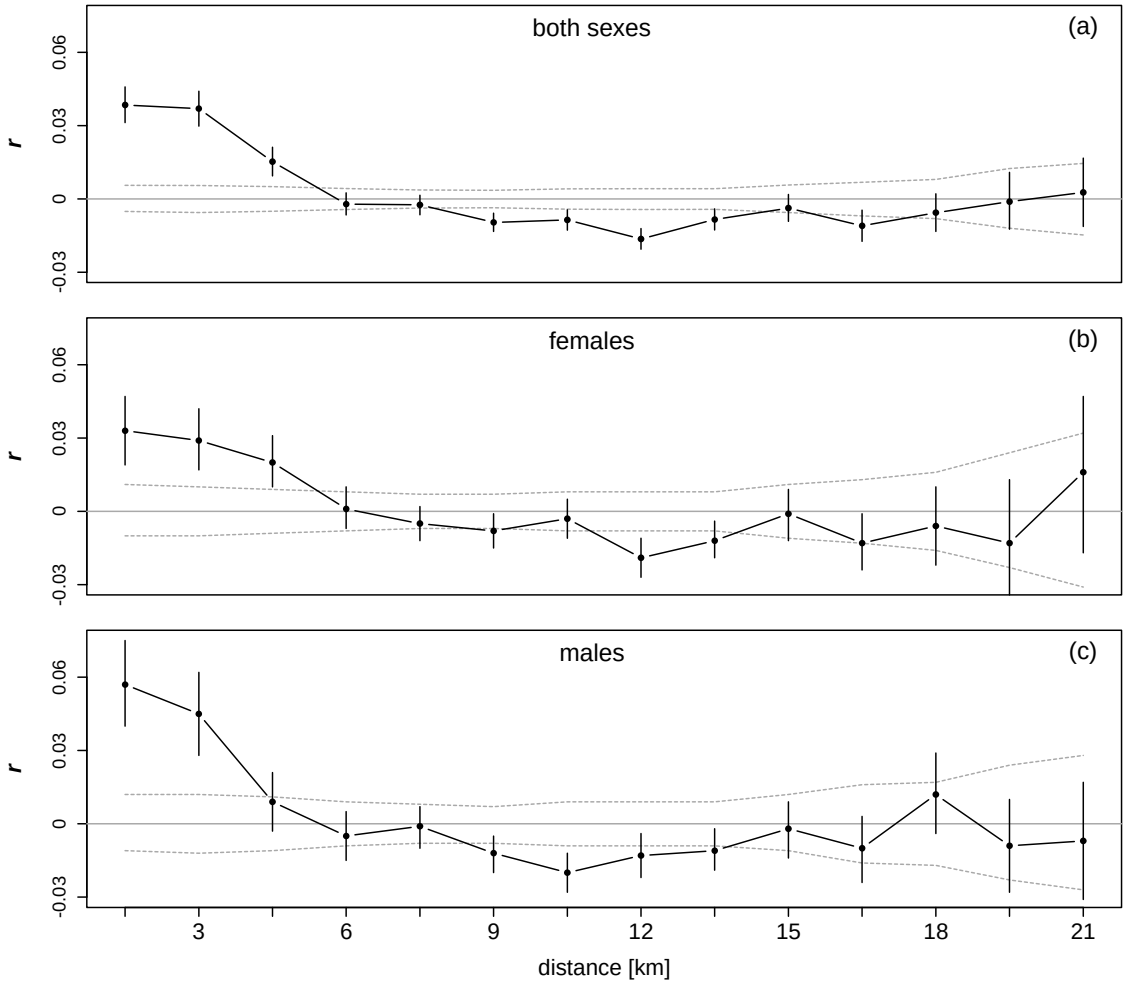

Figure 2: Correlogram plots from spatial genetic autocorrelation between (a) 252 individuals breeding in the study area in different rivers in 2011 and 2013, and separately for the (b) 135 females and (c) 117 males. Autocorrelation coefficients (including their 95% confidence intervals based on 10'000 boostraps) are plotted against geographical distance. Dashed lines indicate the upper and lower 95% confidence interval of no spatial autocorrelation, based on 9999 permutations.

Subsequently, we analysed spatial genetic structure within single populations. In the Sihl river (Fig. 3a), spatial autocorrelation was positive up to 7.2km (r=0) with autocorrelation coefficients being significantly or weakly positive over short distances ($r_{0\text{-}1.5}$=0.018, $r_{1.5\text{-}3.0}$=0.012, and $r_{4.5\text{-}6.0}$=0.010, all p<0.002; $r_{3.0\text{-}4.5}$=0.004, p=0.065) but not over longer distances. In contrast, spatial genetic autocorrelation did not decline with geographical distance in the Küsnacht river (see. Fig. 3b). It should be mentioned that comparisons among non-contemporary individuals were not included in the autocorrelogramm (see methods). Because non-contemporary individuals were genetically more distant (see Fig. S1), spatial genetic autocorrelation for contemporary individuals was on average positive.

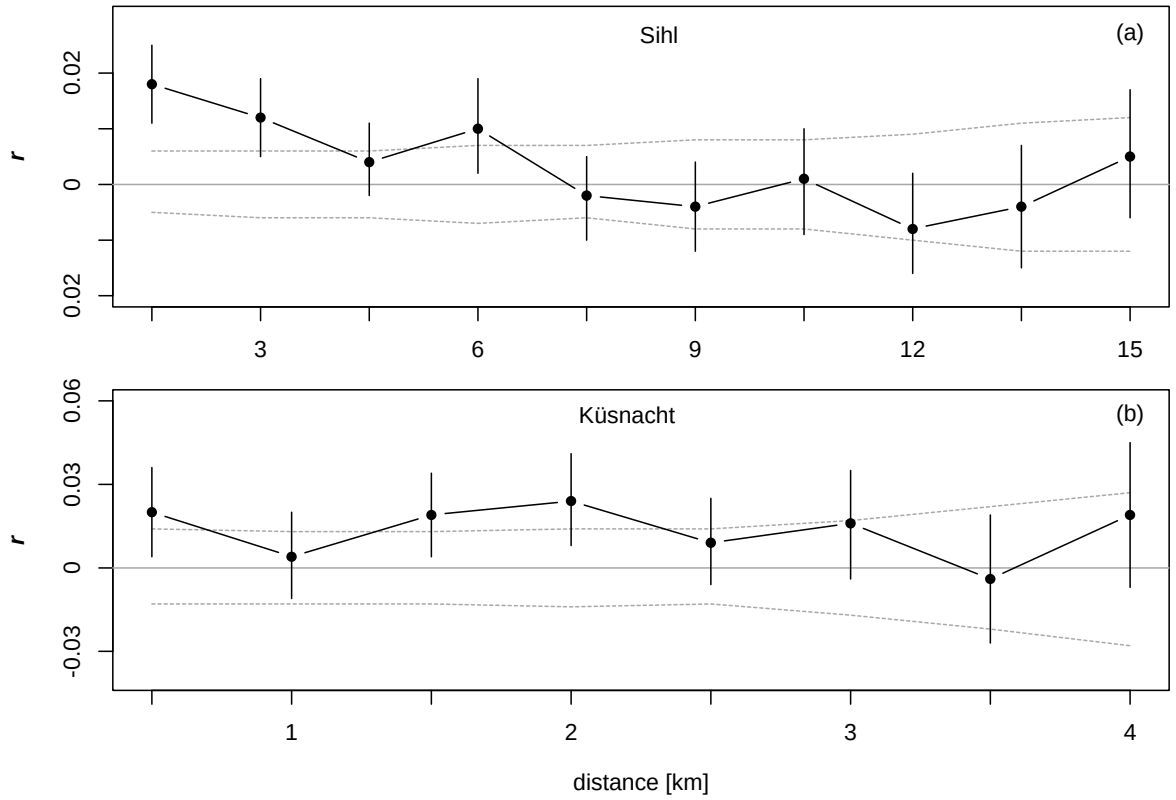

Figure 3: Correlogram plots from spatial genetic autocorrelation between (a) 275 individuals breeding in the Sihl river and (b) 134 individuals breeding in the Küsnacht river between 2004 and 2013. Only individuals that co-occurred in at least one year are compared. Autocorrelation coefficients (including their 95% confidence intervals based on 10'000 boostraps) are plotted against geographical distance. Dashed lines indicate the upper and lower 95% confidence interval of no spatial autocorrelation, based on 9999 permutations.

Patterns of population connectivity and genetic differentiation

The numbers of recruiting individuals in each river and their rivers of origin were extracted from mark-resight data and are summarized in Table S2. Connectivities between each pair of rivers are given in Table 2. All connectivities were smaller than unity, indicating that rivers were to some extent isolated from each other (χ^2=823.77, d.f.=16, p<0.001). Connectivity was on average 0.071 and highest between the rivers Küsnacht and Wehrenbach (0.188). However, connectivities between two populations in both directions (c_{xy} vs. c_{yx}) were not significantly correlated (ρ=0.369, p=0.08), indicating that mutual exchange was not equally strong. Females had higher connectivities ($\bar{c}_{females} = 0.112$, p=0.014) than males ($\bar{c}_{males} = 0.043$), but sex-specific connectivities were correlated (ρ=0.703, p=0.024, Table 3).

Table 2: Connectivity ($c_{x,y}$-matrix) among rivers, based on the total number of individuals that were breeding in five rivers of the study area between 1996 and 2013 (see Fig. S1). As connectivity is a directional measure, $c_{x,y}$ can be different from $c_{y,x}$. For example, connectivity from Küsnacht to Wehrenbach was 0.237, and vice versa only 0.139.

		river y				
		Küsnacht	Wehrenbach	Sihl	Reppisch	Jonen
river x	Küsnacht	-	0.237	0.025	0.056	0.029
	Wehrenbach	0.139	-	0.015	0	0
	Sihl	0.077	0.129	-	0.108	0.252
	Reppisch	0.074	0.072	0.157	-	0
	Jonen	0	0	0.045	0	-

Table 3: Connectivity among rivers, separately for females (above diagonal) and males (below diagonal), based on all females and males, respectively, that were breeding in five rivers of the study area between 1996 and 2013. Connectivities are means of $c_{x,y}$ and $c_{y,x}$ (cf. Table 2).

		$c_{females}$				
		Küsnacht	Wehrenbach	Sihl	Reppisch	Jonen
c_{males}	Küsnacht	-	0.226	0.113	0.139	0
	Wehrenbach	0.163	-	0.177	0.083	0
	Sihl	0.012	0.013	-	0.183	0.197
	Reppisch	0	0	0.090	-	0
	Jonen	0.028	0	0.122	0	-

Using genetic data, we subsequently estimated pairwise population differentiation among rivers in the study area and the populations in the cantons of Jura and Ticino. Both pairwise F_{ST} values and genetic distances D are reported in Table 4. Pairwise F_{ST} values (Weir and Cockerham 1984) among rivers in the study area were between 0.009 and 0.038 (mean 0.020) with eight out of ten values being significantly different from zero after Bonferroni correction (i.e. p<0.00238). Two

pairs of rivers (Küsnacht / Wehrenbach and Sihl / Jonen), being in close proximity to each other and having a high connectivity, were not differentiated after Bonferroni correction (p=0.00813 and 0.00241). Nei's genetic distance D (Nei 1978) among rivers in the study area ranged between 0.031 and 0.132, with a mean distance of 0.069.

Table 4: Genetic distances D (below diagonal, Nei 1978) and pairwise F_{ST}'s (above diagonal, Weir and Cockerham 1984) between five rivers of the study area. F_{ST} values in bold were significant after Bonferroni correction.

		pairwise F_{ST}				
		Kusnacht	Wehrenbach	Sihl	Reppisch	Jonen
genetic distance D	Kusnacht	-	*0.013*	**0.024**	**0.032**	**0.038**
	Wehrenbach	0.041	-	**0.013**	**0.019**	**0.026**
	Sihl	0.081	0.045	-	**0.010**	*0.009*
	Reppisch	0.106	0.065	0.035	-	**0.019**
	Jonen	0.132	0.095	0.031	0.063	-

We examined genetic differentiation also on a larger spatial scale in a hierarchical AMOVA. We grouped all rivers from the study area and included samples from Ticino (125km from Zurich and across the Alps) and Jura (90km from Zurich). As shown by a hierarchical AMOVA, most of the genetic variation (98.04%) was found within rivers. Another 1.90% was among rivers within the study area and only 0.06% of variation was between the three groups. Accordingly, pairwise F_{ST}'s between the five rivers of the Zurich study area and the Ticino group were between 0.012 and 0.032 (mean ± sd = 0.020 ± 0.008). Likewise, birds that were sampled in the canton of Jura were only weakly differentiated from birds in the study area (pairwise F_{ST}'s between 0.017 and 0.028, mean ± sd = 0.022 ± 0.006) and from birds that were sampled in the canton of Ticino (pairwise F_{ST}=0.01238).

As connectivity as defined above measures the (relative) amount of exchange of individuals among rivers, and genetic differentiation describes its genetic consequences, we correlated the two for all rivers within the study area and found they were indeed significantly related (ρ= –0.64, p=0.035 using Mantel test with 10'000 permutations, Fig. 4). Using the preferable linearized F_{ST} (i.e. $F_{ST}/(1-F_{ST})$, Rousset 1997) correlation was slightly higher (ρ= –0.72, p=0.018).

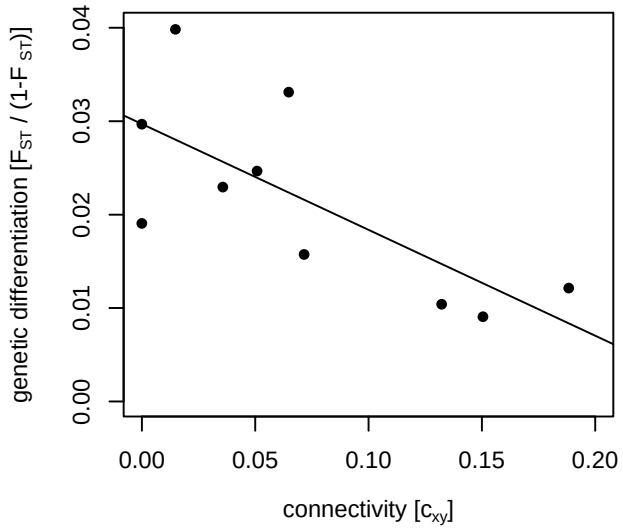

Figure 4: Correlation between population connectivity *c* based on mark-resight data (Table 2) and pairwise population genetic differentiation (F_{ST}, Table 4).

Patterns and probabilities of inbreeding

The average inbreeding coefficient of offspring that hatched between 1996 and 2013 in the Küsnacht, Wehrenbach and Sihl rivers (n=5089) was $f = 0.026$ (Table 5). Of these offspring, 19.9% had at least distantly related parents ($f \geq 0.03125$). Out of those, 48.5% were offspring of close kin, i.e. from matings between half-sibs and full-sibs or between parents and offspring or grandparents and grandchildren ($f \geq 0.125$).

Table 5: Number of offspring and their respective inbreeding coefficients *f*, based on pedigree data and depending on the degree of restrictiveness. The mean inbreeding coefficient (overall *f*) was calculated as the mean of the inbreeding coefficients of all offspring.

	Number of offspring with	
inbreeding coefficient	both parents known	all four grandparents known
$f < 0.03125$	4075	1070
$0.03125 \leq f < 0.0625$	275	270
$0.0625 \leq f < 0.125$	247	247
$0.125 \leq f < 0.25$	337	304
$0.25 \leq f < 0.375$	132	98
$0.375 \leq f < 0.5$	20	20
$f >= 0.5$	3	3
Total	5089	2012
overall *f*	0.026	0.060

Restricting the analysis to offspring with four known grandparents (n=2012) resulted in a higher mean inbreeding coefficient of f = 0.060. Nearly half of these (46.8%) had at least distantly related parents, with 45.1% of them being offspring of close kin. The highest inbreeding coefficient was f = 0.5, resulting from successive close inbreeding.

The probability of pairing with a related individual was strongly dependent on an individual's dispersal behaviour. The proportion of inbred offspring (f≥0.03125) was particularly high for philopatric individuals, with philopatric females pairing with a related mate significantly more often (51.4%; mean inbreeding coefficient in their offspring $\bar{f}_{off}$ =0.053) than philopatric males (32.0%, z=4.00, p<0.001; $\bar{f}_{off}$ =0.034). Dispersal between rivers within the study area led to a strong decline in the probability of producing inbred offspring for females (20.0%, z=3.68, p<0.001; $\bar{f}_{off}$ =0.009), but not for males (28.6%, significant interaction z=2.14, p=0.03; $\bar{f}_{off}$ =0.023). Inbreeding of immigrating individuals can only be detected if they reproduce with one of their descendants (0% in males, $\bar{f}_{off}$=0.0006; 0.5% in females, $\bar{f}_{off}$=0.002).

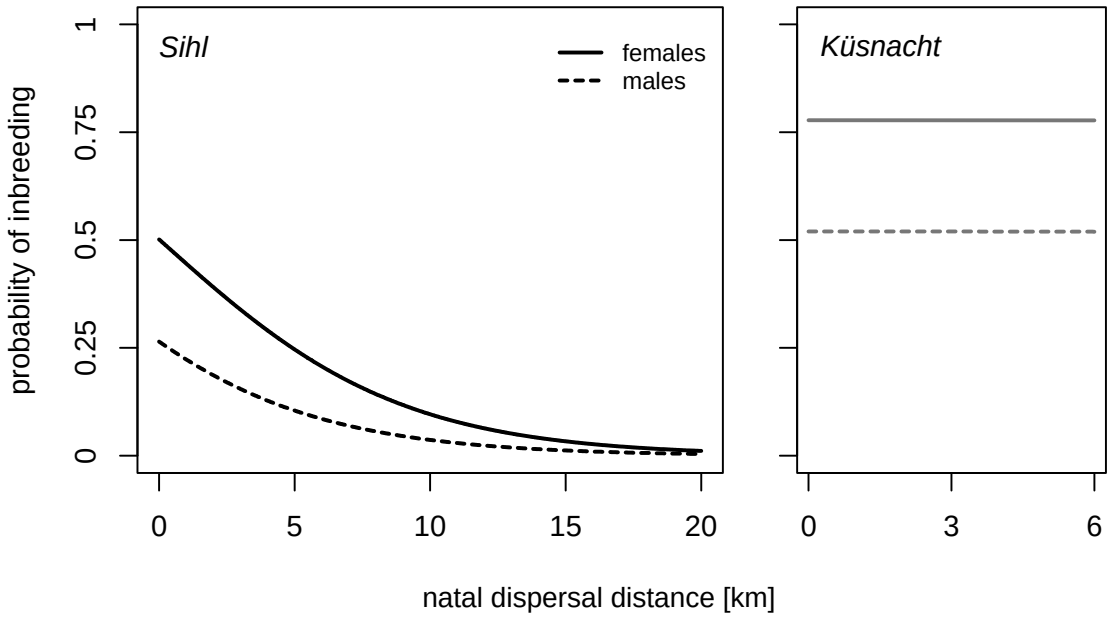

Figure 5: Probability of inbreeding (kinship coefficient ≥0.03125) for philopatric individuals (breeding in their natal river). Females had higher probabilities of mating with a relative than males in both rivers. Natal dispersal distance predicted the probability of inbreeding only in the Sihl river (left panel), but not in the Küsnacht river (right panel).

Subsequently, we analysed the spatial structure of relatedness within two rivers, monitored over 25.5km (Sihl) and 6.5km (Küsnacht) (Fig. 5). For a philopatric female in the Sihl river, the probability of inbreeding when breeding at her natal site (natal dispersal distance=0km) in her year of first reproduction was 50% and decreased significantly ($b=-0.22 \pm 0.08$, $z=-2.69$, $p=0.007$) with distance (25% at 5km distance from the natal site and 10% at 10km). Although probabilities were on average lower for philopatric males (26% at 0km, 10% at 5km and 4% at 10km, estimate = -1.02 ± 0.38, $z=-2.68$, $p=0.007$), they also decreased significantly with distance. This sex difference is a consequence of females being the more dispersive sex. In the shorter river (Küsnacht), probabilities of being related with the mate were independent of the natal dispersal distance ($b=-5.8 \cdot 10^{-4} \pm 0.15$, $z=-0.004$, $p=0.99$) but again higher for females than for males (78% for females and 52% for males; estimate = -1.17 ± 0.42, $z=-2.82$, $p=0.005$).

Discussion

Using mark-resight and genetic data from a long-term study on white-throated dippers, we found frequent inbreeding within rivers, despite weak genetic differentiation among rivers. Despite strong (female-biased) gene flow, individuals that stayed in their natal population showed short-distance dispersal. This resulted in fine-scale spatial genetic structure and high probabilities of inbreeding, in particular in philopatric individuals dispersing over short distances. Because this pattern was much more pronounced in females, it suggests that inbreeding avoidance contributes to maintain a pattern of female-biased dispersal in this species, and probably in other bird species as well.

Short-distance dispersal has been shown in a number of bird species, even in migratory species (e.g. Hansson et al. 2002, Athrey et al. 2012) and highly mobile species like albatrosses (Charmantier et al. 2011). However, when dispersal distances are inferred only from observations of one or few populations within a study area of limited size, dispersal movements away from the study area will usually remain undetected, resulting in an underestimation of the tail end of the dispersal distribution and thereby mean dispersal distance (Van Noordwijk 1984, Koenig et al. 1996). Advances in modern tracking technologies (e.g. using radio telemetry or GPS loggers) allow us to also follow emigrating individuals. Although these studies often have small sample sizes, estimates of dispersal are less likely to be biased and tend to be in good agreement with indirect estimates of dispersal distance based on genetic data (Selonen et al. 2010, Griesser et al. 2014). Using ring recovery data is an alternative approach for obtaining less biased estimates of dispersal (Thomson et al. 2003, Kekkonen et al. 2011). Here, we showed that by only using data from within the study area, we would strongly underestimate mean dispersal distance.

Estimates of connectivity between populations based on mark-resight data showed that populations were indeed isolated from each other to some degree. Furthermore, the variation in the amount of exchange among populations was correlated with patterns of pairwise genetic differentiation, even though genetic differentiation was on average weak. Weak or no genetic differentiation across large spatial scales has also been found in other sedentary bird species, including great tits, house sparrows and blackbirds (Partecke et al. 2006, Pavlova et al. 2006, Postma et al. 2009, Kekkonen et al. 2011), which is at least partly the result of the continuity of their habitat and potentially high mobility. Remarkably, in dippers genetic differentiation between distant populations was in some cases weaker than between populations in spatial proximity, with especially the Küsnacht population being particularly isolated, as is obvious from its low rates of immigration. Similarly, Postma et al. (2009) showed strong differentiation between two populations of great tits on the same island but only weak differentiation of one of these island

populations to a distant mainland population, a pattern which was maintained by different levels of gene flow from the mainland into the two island populations (Postma and Van Noordwijk 2005).

Despite weak genetic differentiation on the population scale, spatial autocorrelation analysis revealed significant small-scale spatial genetic structure. It should be noted however that this analysis assumes the habitat is continuous, which is not the case here. Therefore, the spatial analysis over the entire study area may partly be driven by between-river differentiation. Nevertheless, when we limit ourselves to a single river, a similar albeit less strong pattern emerges at least in the longest of our study rivers (Fig. 3). Furthermore, despite female-biased dispersal, both sexes show fine-scale genetic structure albeit stronger in males. This is in contrast to two other studies using spatial autocorrelation analysis for inference about sex-biased dispersal, which found that the dispersive sex did not contribute to genetic structure (Athrey et al. 2012, Banks and Peakall 2012).

Although small-scale genetic structure within areas due to limited dispersal has been repeatedly shown (see references before), the gene pool will typically be mixed completely across generations, Therefore, genetic structure within populations is of little interest from a population genetic perspective (e.g. Van Tienderen and Van Noordwijk 1988, but see Garant et al. 2005). However, here we have shown that genetic structure plays a major role in shaping levels of inbreeding. In the Sihl river (25.5km long), probabilities of inbreeding increased with decreasing natal dispersal distance of philopatric individuals in a very similar pattern as positive autocorrelation in genetic structure increased (Fig. 3b and Fig. 5). Probabilities of inbreeding were even higher in the shorter Küsnacht river (Fig. 5), which is in accordance with a high proportion of philopatric individuals and the relatively small number of breeding pairs (13 on average). Assuming no additional mechanisms of inbreeding avoidance, this suggests that inbreeding is common in philopatric individuals, in particular if they disperse over short distances within their natal river. Indeed, we found that many philopatric individuals mated with a relative, leading to high levels of inbreeding. This is in line with data on great tits, in which natal dispersal distance has been shown to be a major predictor for the relatedness of mates, in particular in females (Van de Casteele and Matthysen 2006, Szulkin and Sheldon 2008). Stronger effects in philopatric females, as also found in our study, are a consequence of female-biased dispersal. In turn, female-biased probabilities of inbreeding in philopatric individuals might contribute to the maintenance of female-biased dispersal in this species, and probably in other bird species as well.

Overall, our data provide good evidence for natal dispersal being of high importance for inbreeding avoidance, especially given the fact that numerous studies do not find evidence for the

existence of other mechanisms (Keller and Arcese 1998, Wheelwright and Mauck 1998, Hansson et al. 2006, Szulkin and Sheldon 2008, but see Wang and Lu 2011, Billing et al. 2012, Olson et al. 2012).

Here we showed that inbreeding can still be common if dispersal is limited, and in particular as the breeding habitat is linear and populations are small. Inferring levels of inbreeding from all individuals that hatched within the study area (mean *f* of 0.026) is likely to even underestimate true inbreeding. This is because immigrating individuals might be related to their mate (though unlikely) and thus have inbred offspring. On the contrary, restricting the analysis to offspring with four known grandparents (mean *f* of 0.060) will exclude all truly non-inbred offspring with at least one immigrating parent and thus overestimate mean inbreeding. Yet it is striking that the level of inbreeding in an isolated island population of song sparrows (*Melospiza melodia*) (Reid et al. 2014) is probably only twice as high as in our system with high rates of gene flow. In great tits (*Parus major*), on the other hand, in which rates of dispersal are comparable to those we observe for white-throated dippers, mean inbreeding coefficients were found to be substantially lower (0.00273; Szulkin et al. 2007). This highlights that limited dispersal in fragmented environments can lead to high levels of inbreeding.

In conclusion, we have analysed dispersal behaviour and genetic structure, both on the within- and the between-population level. We have shown that high levels of inbreeding, in particular for philopatric females, are the result of a high proportion of philopatric individuals showing short-distance dispersal and of female-biased dispersal. The fragmented and linear nature of the breeding habitat and small population sizes contribute to high levels of inbreeding. In contrast to these small-scale patterns, genetic differentiation on the larger scale was weak. This implies that measures of weak genetic differentiation among populations do not exclude the frequent occurrence of inbreeding within populations. This may be the case for many species and populations living in fragmented habitat, and is of particular relevance for species of conservation concern.

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Appendix

Table S1: Number of samples (n) for calculation of the standardized number of alleles per locus (sNa) and observed (H_o) and expected (H_e) heterozygosity in seven rivers in the cantons of Zurich (5), Ticino (1) and Jura (1). Samples from Zurich are of individuals breeding in 2012 and 2013, and samples in Ticino and Jura were collected during the early breeding season in 2013.

canton	*river*	*n*	*sNa*	H_o	H_e
Zurich	*Küsnacht*	46	7.29	0.74 ± 0.16	0.75 ± 0.14
	Sihl	82	8.09	0.78 ± 0.13	0.78 ± 0.12
	Wehrenbach	19	7.75	0.77 ± 0.14	0.77 ± 0.14
	Reppisch	26	7.49	0.75 ± 0.16	0.76 ± 0.15
	Jonen	24	7.80	0.81 ± 0.14	0.78 ± 0.13
Ticino	*Maggia*	32	8.69	0.79 ± 0.12	0.79 ± 0.12
Jura	*La Sorne/Scheulte*	30	8.12	0.80 ± 0.14	0.78 ± 0.14

Table S2: Natal dispersal. Number of individuals recruiting (breeding) in five rivers of the study area between 1996 and 2013 in relation to their river of birth. Individuals originating from "Other" are either birds that were ringed in one of the smaller rivers of the study area or immigrants with unknown origin.

		river of breeding				
		Küsnacht	Wehrenbach	Sihl	Reppisch	Jonen
river of birth	Küsnacht	132	16	7	3	1
	Wehrenbach	9	33	2	0	0
	Sihl	7	6	193	4	6
	Reppisch	2	1	9	11	0
	Jonen	0	0	4	0	11
	Other	42	42	191	60	32

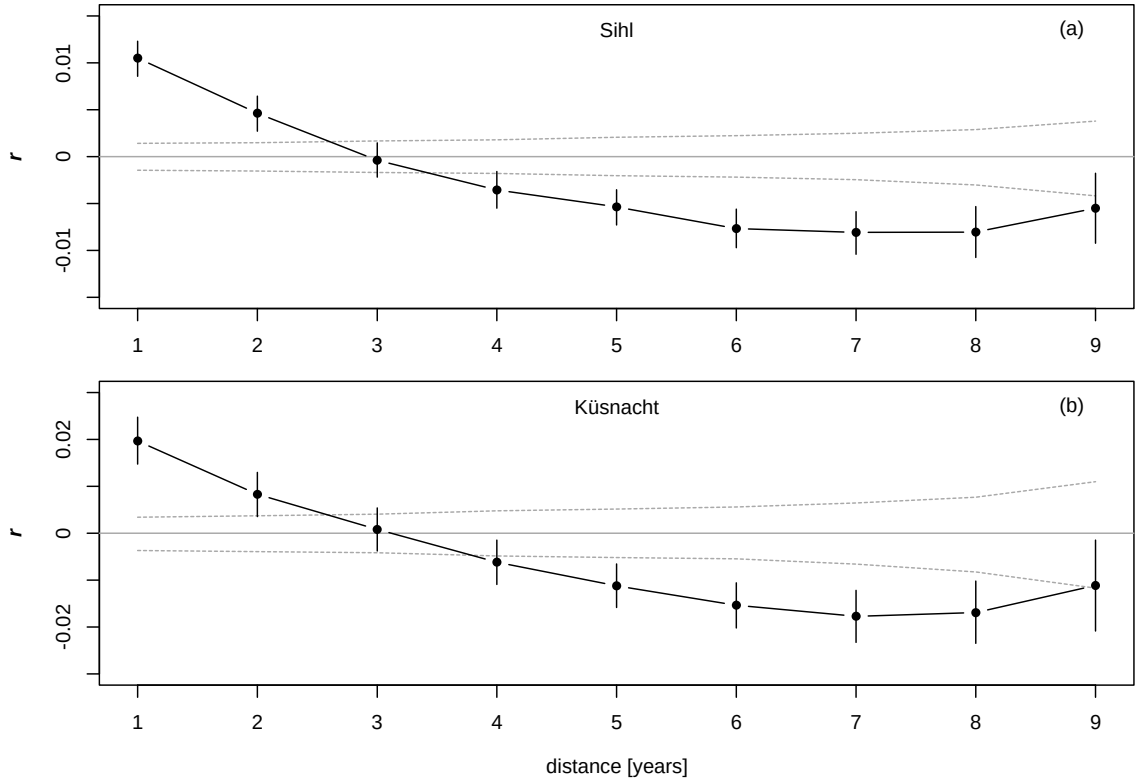

Figure S1: Correlogram plots of "temporal genetic autocorrelation" between (a) 598 individuals breeding in the Sihl river and (b) 296 individuals breeding in the Küsnacht river between 2004 and 2013. Autocorrelation coefficients (including their 95% confidence intervals based on 10'000 boostraps) are plotted against temporal distance, measured as the number of years between recruitment of two individuals. For example, two individuals that reproduced in 2006 and 2008 for the first time, respectively, have a distance of two years. Dashed lines indicate the upper and lower 95% confidence interval of no autocorrelation, based on 9999 permutations.

Chapter 4

Phenotype-associated inbreeding biases estimates of inbreeding depression in a wild bird population

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Abstract

Inbreeding depression is usually quantified by regressing individual phenotypic values against inbreeding coefficients, implicitly assuming there is no correlation between phenotypes and relatedness of mates. However, if the occurrence of inbreeding is associated with the phenotype or if dispersal is phenotype-dependent, this assumption might be violated. Here we show that such an association can severely bias estimates of inbreeding effects. We do this using a long-term individual-based data set from white-throated dippers (*Cinclus cinclus*), a bird species in which inbreeding is relatively common. We show that during part of the study period, parents of inbred birds had shorter wings than those of outbred birds, and as wing length is heritable, inbred individuals were smaller, independent of any inbreeding effects. This resulted in the overestimation of inbreeding effects. Similarly, during a period when parents of inbred birds had longer wings, we found that inbreeding effects were underestimated. We discuss how such associations may have arisen in this system, and why they are likely to be common in others, too. Overall, we highlight the importance of simultaneously accounting for inbreeding and additive genetic effects and demonstrate how unbiased estimates of inbreeding depression can be obtained within a quantitative genetic framework.

Keywords: white-throated dipper · inbreeding · animal model · phenotype-dependent dispersal · heritability

Introduction

In spatially fragmented environments, especially if populations are small and isolated, inbreeding can be a common phenomenon (Lande 1988, Keller 1998). As it increases homozygositiy, inbreeding results in deleterious recessive mutations being expressed with higher probability, a reduction in the frequency of heterozygotes at loci showing overdominance, and/or changes in gene interactions, all of which may negatively affect trait values and fitness (Crow and Kimura 1970, p 78-80). Since Darwin (1876), numerous studies testing for negative consequences of inbreeding (i.e. inbreeding depression) have shown that inbreeding depression is common, both in captive and wild populations (Charlesworth and Charlesworth 1987, Keller and Waller 2002). In addition to individual-level effects on a variety of traits like body mass, survival, and fecundity in a range of plant and animal species, including humans, (e.g. DeRose and Roff 1999, Richards 2000, Kruuk et al. 2002, Szulkin et al. 2007, Postma et al. 2010, Hemmings et al. 2012) (but see Duarte et al. 2003, Thünken et al. 2007), the fitness-related consequences of inbreeding may also have population-level consequences, threatening the persistence of small populations (Heschel and Paige 1995, Nieminen et al. 2001).

The degree of inbreeding of an individual is measured as its coefficient of inbreeding, i.e. the probability of two alleles being identical by descent (IBD) (Wright's inbreeding coefficient f; Wright 1922, Malécot 1948). Inbreeding coefficients can be obtained from pedigree data, in which case they are estimated relative to a base population consisting of unrelated founders and immigrants. Alternatively, inbreeding coefficients can be inferred from multi-locus genotype data (see Balloux et al. 2004, Slate et al. 2004, and Bérénos et al. 2014 for evaluations of marker-based inbreeding estimates) for evaluations of marker-based inbreeding estimates). Having a (pedigree- or marker-based) measure of inbreeding for each individual, we can test for inbreeding depression by regressing phenotypic values on individual inbreeding coefficients in a linear (mixed) model framework. Such analyses typically show that inbred individuals have smaller trait values than outbred ones (with few exceptions like laying date in birds, where smaller values are typically associated with higher fitness; Gienapp et al. 2006, Keller et al. 2006). Here it should be noted that although historically the term inbreeding depression has been used to refer to traits that are more or less closely linked to fitness, here we use it to describe any relationship between inbreeding and phenotype, irrespective of its effect on fitness.

Inferring inbreeding depression from the relationship between phenotype and inbreeding coefficient assumes that inbreeding individuals are a random subsample of the population with respect to the trait of interest. In other words, it assumes that there is no correlation between an individual’s phenotype and the kinship coefficient to its mate, i.e. the inbreeding coefficient of its

offspring. However, if, for example, related mates are characterized by lower trait values than unrelated ones, inbred offspring will have parents with lower trait values. If the trait is heritable, inbred offspring will be characterized by small trait values not only due to the potential effects of inbreeding, but also because of the additive genetic effects passed on by their parents. Hence, if additive genetic effects are not accounted for, this scenario will result in an overestimation of the magnitude of inbreeding depression. Likewise, the magnitude of inbreeding depression may be underestimated if inbred offspring have parents with higher trait values.

Although the above scenario has been hypothesised before (Lynch and Walsh 1998, p. 270-272 , Reid et al. 2008), we are not aware of empirical studies that have tried to quantify the effects of phenotype-associated inbreeding. This is to some degree surprising, as there is in fact abundant evidence for phenotype-associated inbreeding. For example, in an island population of song sparrows (*Melospiza melodia*), males with specific phenotypes (like earlier hatching date, shorter tarsi, and lower survival probability) paired with close relatives more often than expected by chance (Reid et al. 2008). Similarly, in collared flycatchers (*Ficedula albicollis*) offspring are more often inbred in the late breeding season than earlier in the season (Kruuk et al. 2002), and in Seychelles warblers (*Acrocephalus sechellensis*) subordinate females mate with relatives more often than expected by chance (Richardson et al. 2004).

Such associations can arise for different reasons (see also Reid et al. 2008). For example, individuals of higher quality might be able to avoid inbreeding more effectively than individuals of lower quality (e.g. Richardson et al. 2004). Alternatively, given high philopatry and random mate choice, individuals with many siblings (and therefore a higher breeding value for fecundity) have a higher probability of pairing with a sib (Van Noordwijk and Scharloo 1981). Furthermore, phenotype-associated inbreeding can be generated by phenotype-dependent dispersal, as dispersers typically do have lower probabilities of inbreeding than philopatric individuals (e.g. Szulkin and Sheldon 2008) and differ phenotypically from the latter. For example in birds and many insects, dispersal behaviour is a function of body size, especially wing length, with bigger or longer winged individuals dispersing further (Paradis et al. 1998, Skjelseth et al. 2007, Dawideit et al. 2009) (but see Chaput-Bardy et al. 2010). In line with this, differences between philopatric individuals and dispersers have been detected in a range of morphological, behavioural and life-history traits, and in taxa ranging from single-cell species to primates (reviewed in Ronce and Clobert 2012). In summary, estimates of inbreeding depression may be biased even under very general conditions.

Here we provide an empirical test of a scenario of phenotype-associated inbreeding, using a long-term individual-based data set for white-throated dippers (*Cinclus cinclus*), a bird species living exclusively along rivers. We take wing length, which in this species is associated with dispersal behaviour, as our trait of interest and ask whether an individual's wing length is correlated with the kinship coefficient with its mate. Subsequently, we test whether such a correlation between phenotype and kinship results in a biased estimate of inbreeding effects. We show that by using a quantitative genetic animal model, which explicitly accounts for additive genetic differences between inbred and outbred offspring, we are able to obtain unbiased estimates of inbreeding depression.

2. Material and Methods

Study system and data set

The white-throated dipper is a medium-sized passerine (in our study population males are 62.5 ± 3.6g and females 53.7 ± 4.1g; mean ± s.d.) that is widely distributed across Europe. It lives along streams and rivers and mainly feeds on aquatic invertebrates. After an incubation time of 16-17 days, offspring of the first brood (brood size at ringing: 4.4 ± 1.1 nestlings) hatch between the middle of March and the beginning of May. About 35% of all offspring are from second broods (Hegelbach 2013) with 3.7 ± 1.1 nestlings hatching between the end of April and the beginning of June. Both parents provide food to the offspring, which fledge 21-24 days after hatching.

Since 1987, dippers of the subspecies *C. c. aquaticus* have been monitored intensively at eleven rivers spanning an area of approximately 400km^2 in the proximity of Zurich, Switzerland (see Fig. 1 in Chapter 1 for a map). Here, we used data from the Küsnacht (river length: 6.5km), Sihl (25.5km), and Wehrenbach (5.5km) rivers. In these rivers, more than 99% of all broods could be accessed and virtually all breeding individuals were known (between 1996 and 2013 only 0.1% and 0.5% of ringed nestlings had an unknown mother or father, respectively). Adults without rings (i.e. immigrants) were captured to be colour-ringed and measured usually before the breeding season, but at the latest before their offspring were ringed. Offspring were colour-ringed and measured at an age of 9-14 days.

Behavioural observations during the mating and breeding season allowed determining parentage of each brood. Because dippers have a very low rate of extra-pair paternity (2% according to Øigarden et al. 2010; less than 1% according to our own unpublished data), we reconstructed the pedigree based on behavioural observations. We calculated Wright's inbreeding coefficient (*f*) for all individuals since 1987 using the software Pedigree Viewer (available at http://www.personal.une.edu.au/~bkinghor/pedigree.htm). Because founders and immigrants by definition have unknown parents and are therefore assigned an (uninformative) inbreeding coefficient of zero, all analyses are restricted to individuals that have hatched in the study area. We excluded individuals hatched in 1995 or earlier because monitoring was still incomplete in the first years of the study and levels of inbreeding might thus be underestimated. The mean inbreeding coefficients of individuals from the cohorts 1996-2013 was 0.026 ± 0.063 (mean ± s.d., max. = 0.5).

Independent and fully-grown offspring were recaptured using mist nets to measure, amongst others, wing length (to the nearest 0.5mm). Since 2008, the state of the primary feathers was scored as worn or not worn, with worn feathers being shorter. In a first step, we therefore confined

our analyses to individuals with known feather state, resulting in 192 individuals of the six cohorts that hatched from 2008 to 2013. Subsequently, we replicated the analyses with data for birds that hatched between 1996 and 2007, to test whether patterns were alike or different in these years, and the generality of our predictions regarding the potential bias on estimates of inbreeding effects. To this end, we divided these earlier cohorts into two periods of six years (251 individuals from the cohorts 1996-2001 and 229 individuals from the cohorts 2002-2007).

Statistical analyses

First, we tested whether inbreeding occurred randomly with respect to wing length by correlating the wing length of parents (mid-parent values) with their coefficient of kinship (i.e. the inbreeding coefficient of their offspring) using a Spearman rank correlation. Here we considered only parents of individuals used to estimate inbreeding depression later on. We did this separately for the three above mentioned periods, each containing six cohorts.

We subsequently estimated the effect of inbreeding on wing length, again separately for the three time periods. We first did this using the standard method of fitting a linear mixed effect model, including sex, natal population and the state of the feathers as fixed effects, and the inbreeding coefficient as a covariate. Because feathers often become longer with age in birds (e.g. Alatalo et al. 1984), we also fitted the age at measurement (in years) as a covariate. Cohort (i.e. year of birth) and individual's identity (ID) were included as random effects to account for random environmental variability among years and for multiple measurements per individual, respectively. In this model, the random individual effect captures the variance due to both permanent environment and (additive and non-additive) genetic effects.

In a final step, we extended the mixed model outlined above to an animal model (Kruuk 2004, Wilson et al. 2010) by additionally fitting an additional random additive genetic (animal) effect. This animal effect estimates the variance in the trait, i.e. in wing length, that is due to additive genetic effects, using information on the relatedness and resemblance in wing length among all individuals in the pedigree. Not only does this allow for the estimation of the narrow-sense heritability (h^2; the proportion of phenotypic variance explained by additive genetic effects) of wing length, but in this context most importantly, it enabled us to separate additive genetic effects from the effect of inbreeding. If inbreeding is associated with the phenotype, we expect the estimate of the inbreeding effect to differ between the animal model and the standard mixed effect model described before.

Statistical analyses were performed using R version 3.0.2 (R Development Core Team 2013). Mixed effect models without and with an animal effect were fitted using restricted maximum likelihood (REML) in ASReml version 3.0 (Gilmour et al. 2009). Statistical significance of fixed and random effects was assessed using conditional Wald F-tests and likelihood ratio tests, respectively.

Results

We first restricted ourselves to the cohorts 2008-2013, because information on the state of the feathers was collected since 2008. During this period, parents were more related to each when they had shorter wings (rho=-0.26, p<0.001). From the perspective of the offspring, inbred offspring had parents with shorter wings than outbred offspring.

Without accounting for additive genetic effects (Table 1a), we found that wing length is highly sexually dimorphic, with males having 7.3 mm (± 0.22mm, $F_{1,198.0}$=1104.49, p<0.001) longer wings than females. Furthermore, wing length increased slightly but significantly with age (0.43 ± 0.08mm, $F_{1,154.1}$=25.55, p<0.001), and wings were shorter when feathers were worn (-0.78 ± 0.18mm, $F_{1,217.9}$=19.32, p<0.001). Most importantly, wing length decreased significantly with increasing inbreeding coefficient (-5.58 ± 1.63mm, $F_{1,175.5}$=11.72, p<0.001), i.e. inbred birds had shorter wings. The variance component for individual identity, combining permanent environment and genetic effects, was 1.57 ± 0.26 (χ^2=60.47, p<0.001), explaining 62.4 ± 6.2% of the variance in wing length after accounting for the variance explained by the fixed effects. Variation in wing length among cohorts was negligible ($1.37 \times 10^{-2} \pm 5.89 \times 10^{-2}$, χ^2=0.06, p=0.41).

Expanding the above-described mixed model to an animal model to account for additive genetic resemblance among parents and offspring (Table. 1b), the additive genetic variance was estimated as 1.59 ± 0.48 (χ^2=21.97, p<0.001), providing an estimate of heritability for wing length of 58.8 ± 13.1%. In line with this result, while the variance due to individual identity (here only describing the permanent environment) dropped to 0.21 ± 0.30, explaining another 7.8% of the phenotypic variance (χ^2=0.62, p=0.22). Whereas estimates for the effects of sex, age and the state of the feathers were close to those of the mixed model without an additive genetic animal effect, the estimate for the effect of the inbreeding coefficient was now only -3.52 ± 1.93mm ($F_{1,164.6}$=3.34, p=0.07), corresponding to a decline of 37% in the magnitude of the inbreeding effect (Fig. 1).

As the exclusion of this covariate did not have a major effect on estimates of inbreeding depression in the models described above, we explored in a next step the patterns in the previous cohorts, for which no information on the state of the feathers was available. Contrary to the period 2008-2013 (see above), kinship coefficients increased with increasing (mid-parent) wing length in the first period (1996-2001) (rho=0.13, p=0.04). Thus, inbred individuals had parents with longer wings compared to outbred individuals. In the second period (2002-2007), inbreeding was independent of parental wing length (rho=-0.06, p=0.41).

As before, we analysed variation in wing length using both standard mixed effect models and animal models, accounting for additive genetic effects. Variance component estimates for the

permanent environment, common environment and additive genetic effects are given in Table 1. The estimate for the difference between males and females was similar over the entire study period and irrespective of the model used (standard mixed vs. animal model). Irrespective of which model was used, the effect of age was smaller in the first cohorts (1996-2001; standard mixed model estimate: 0.13 ± 0.04, $F_{1,330.8}$=11.20, p<0.001; animal model estimate: 0.14 ± 0.04, $F_{1,349.9}$=14.36, p<0.001) compared to later cohorts (see above and Table. 1).

In line with the differences in the associations between wing length and kinship coefficients in parents between these three periods, accounting for additive genetic effects changed estimates of inbreeding effects in different ways. In the first period, when related parents had longer wings, the point estimate for the effect of inbreeding was higher from the animal model than from the standard mixed effect model (-2.9 ± 1.8mm, $F_{1,179.1}$=2.48, p=0.12 versus -0.74 ± 1.32mm, $F_{1,255.9}$=0.31, p=0.58, respectively, Figure 1). In the second period, when we detected no association between wing length and kinship coefficients in parents, the effect of the inbreeding coefficient on wing length changed only marginally between the two types of models (standard mixed model: +2.12 ± 1.83, $F_{1,225.5}$=1.34, p=0.25; animal model: +2.74 ± 2.22mm, $F_{1,205.2}$=1.52, p=0.22) and was in fact positive (Fig. 1).

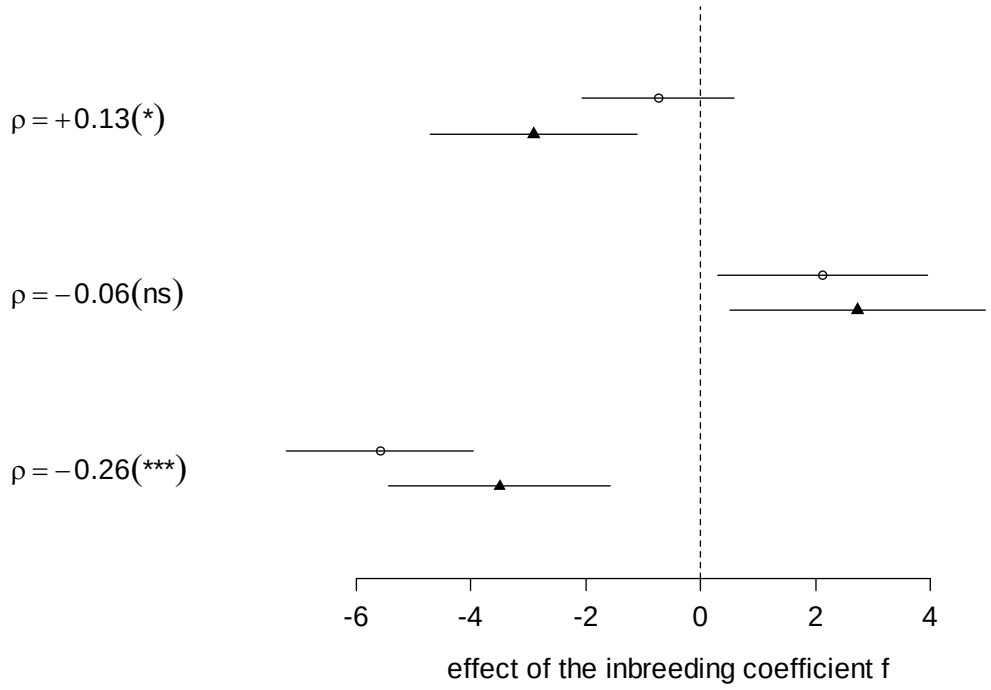

Figure 1: Effect of the inbreeding coefficient f on wing length (mean ± s.e.), based on standard linear mixed effect models (open circles) and animal models (closed triangles), respectively. If additive genetic effects were not accounted for, inbreeding effects were underestimated when the correlation between kinship coefficient and wing length in parents was positive (top; cohorts 1996-2001) and overestimated when the correlation was negative (bottom; cohorts 2008-2013). No bias occurred, when there was no correlation (middle; cohorts 2002-2007).

Table 1: Analysis of variance in wing length in white-throated dippers. Estimates for fixed and random effects from standard linear mixed effect models are given in (a), those from animal models in (b). Inbreeding coefficient (*f*), sex, age (in years) and the state of the wing (feathers worn or not) were included as fixed effects, individual identity (ID) and cohort (year) as random effects. Animal models furthermore estimate additive genetic variance (animal). Data were analysed separately for the cohorts 1996-2001, 2002-2007 and 2008-2013.

***(a)** standard mixed model*	**1996-2001**			**2002-2007**			**2008-2013**		
fixed effect	**estimate ± s.e**	**test statistic**	**p-value**	**estimate ± s.e**	**test statistic**	**p-value**	**estimate ± s.e**	**test statistic**	**p-value**
intercept	92.47 ± 0.16			92.08 ± 0.21			91.91 ± 0.21		
f	-0.74 ± 1.32	$F_{1,255.9} = 0.31$	0.58	2.12 ± 1.83	$F_{1,225.5} = 1.34$	0.25	-5.58 ± 1.63	$F_{1,175.5} = 11.72$	<0.001
sex (female)	-7.75 ± 0.20	$F_{1,241.6} = 1580.82$	<0.001	-7.80 ± 0.20	$F_{1,223.9} = 1575.60$	<0.001	-7.32 ± 0.22	$F_{1,198.0} = 1104.49$	<0.001
age	0.13 ± 0.04	$F_{1,330.8} = 11.20$	<0.001	0.36 ± 0.04	$F_{1,311.4} = 65.49$	<0.001	0.43 ± 0.08	$F_{1,154.1} = 25.55$	<0.001
wing (worn)	-	-	-	-	-	-	-0.78 ± 0.18	$F_{1,217.9} = 19.32$	<0.001
random effect	**variance ± s.e.**	**test statistic**	**p-value**	**variance ± s.e.**	**test statistic**	**p-value**	**variance ± s.e.**	**test statistic**	**p-value**
animal (V_A)	-	-	-	-	-	-	-	-	-
individual (V_{ID})	1.54 ± 0.21	$\chi^2 = 125.6$	<0.001	1.27 ± 0.21	$\chi^2 = 72.9$	<0.001	1.57 ± 0.26	$\chi^2 = 60.47$	<0.001
cohort (V_{YEAR})	0.03 ± 0.06	$\chi^2 = 0.6$	0.22	0.11 ± 0.11	$\chi^2 = 4.4$	0.02	0.01 ± 0.06	$\chi^2 = 0.06$	0.40
residual (V_R)	0.97 ± 0.09			1.26 ± 0.13			0.90 ± 0.13		

***(b)** animal model*	**1996-2001**			**2002-2007**			**2008-2013**		
fixed effect	**estimate ± s.e**	**test statistic**	**p-value**	**estimate ± s.e**	**test statistic**	**p-value**	**estimate ± s.e**	**test statistic**	**p-value**
intercept	92.46 ± 0.25			92.12 ± 0.25			91.80 ± 0.28		
f	-2.91 ± 1.85	$F_{1,179.1} = 2.48$	0.12	2.74 ± 2.22	$F_{1,205.2} = 1.52$	0.22	-3.52 ± 1.93	$F_{1,164.6} = 3.34$	0.07
sex (female)	-7.71 ± 0.18	$F_{1,208.4} = 1908.63$	<0.001	-7.79 ± 0.18	$F_{1,209.8} = 1795.14$	<0.001	-7.29 ± 0.20	$F_{1,191.3} = 1299.19$	<0.001
age	0.14 ± 0.04	$F_{1,349.9} = 14.36$	<0.001	0.34 ± 0.04	$F_{1,326.0} = 61.48$	<0.001	0.40 ± 0.08	$F_{1,171.1} = 25.01$	<0.001
wing (worn)	-	-	-	-	-	-	-0.71 ± 0.17	$F_{1,221.8} = 16.66$	<0.001
random effect	**variance ± s.e.**	**test statistic**	**p-value**	**variance ± s.e.**	**test statistic**	**p-value**	**variance ± s.e.**	**test statistic**	**p-value**
animal (V_A)	1.36 ± 0.44	$\chi^2 = 21.2$	<0.001	1.18 ± 0.37	$\chi^2 = 19.7$	<0.001	1.59 ± 0.48	$\chi^2 = 22.0$	<0.001
individual (V_{ID})	0.33 ± 0.28	$\chi^2 = 1.2$	0.14	0.17 ± 0.26	$\chi^2 = 0.5$	0.24	0.21 ± 0.30	$\chi^2 = 0.6$	0.22
cohort (V_{YEAR})	0.06 ± 0.07	$\chi^2 = 2.0$	0.08	0.11 ± 0.10	$\chi^2 = 5.2$	0.01	$8x10^{-8} \pm 1x10^{-8}$	$\chi^2 = 0$	0.5
residual (V_R)	0.98 ± 0.09			1.26 ± 0.13			0.90 ± 0.13		

Discussion

Here we used a long-term data set on white-throated dippers to investigate whether phenotype-associated inbreeding biases estimates of inbreeding depression, illustrated using data on wing length.

In our study population, dispersal behaviour is dependent on wing length, with dispersing individuals having on average longer wings (+0.33 ± 0.12, t=2.62, p=0.009, unpublished data) than philopatric individuals, i.e. individuals that stay in their natal river. Similar size-dependent dispersal behaviour has been shown in a range of species (Paradis et al. 1998, Skjelseth et al. 2007, Dawideit et al. 2009) (but see Chaput-Bardy et al. 2010). This, combined with typically higher probabilities of inbreeding in philopatric individuals (Szulkin and Sheldon 2008), has the potential to generate a negative association between wing length and the probability of inbreeding.

Indeed, during the last six years of the study (2008-2013), inbred individuals had shorter-winged parents. Similarly, in song sparrows, males with shorter tarsi paired with more closely related mates (Reid et al. 2008). Interestingly however, the relationship between wing length and the kinship coefficients of parents (i.e. the inbreeding coefficient of their offspring) deviated from our expectation in the first two periods. Although inbreeding was again phenotype-associated in the first period (1996-2001), inbred offspring had longer-winged parents instead. In the second period (2002-2007) inbreeding occurred randomly with respect to wing length. Such heterogeneous patterns might result from differences in mate availability between the different periods, or from variation in patterns of mate choice with respect to relatedness, wing length, or some other trait. Alternatively, mate choice might be under the control of inbreeding avoidance. If, for example, costs of inbreeding differ in time, optimal mate choice with respect to kinship and correlated phenotypes might vary likewise (compare Van Noordwijk and Scharloo 1981, Keller and Arcese 1998, Reid et al. 2006). Although worthy of further investigation, conclusively identifying the mechanism responsible for generating phenotype-associated inbreeding, and in particular for variation in this relationship across time, is beyond the scope of this study.

Irrespective of the underlying mechanism, we were able to directly quantify the influence of phenotype-associated inbreeding on estimates of inbreeding effects. To do so, we compared estimates of inbreeding effects from a standard linear mixed effect model with those of an animal model, which explicitly accounts for phenotypic resemblance among related individuals due to shared additive genetic effects. In line with our predictions, inbreeding effects were underestimated when inbred individuals had longer-winged parents, unbiased if inbred individuals had parents of average wing length, and overestimated if inbred individuals had shorter-winged

parents. The possibility that inbreeding effects may not only be over- but also underestimated was already suggested by Van Noordwijk and Scharloo (1981), who showed in a population of great tits that inbreeding individuals produced a higher number of recruiting offspring than those with unrelated mates. Provided additive genetic variation for fitness, those inbred offspring will inherit the genes for producing many recruiting offspring, resulting in an underestimation of the effect of inbreeding on reproductive success.

Not only did the strength and direction of phenotype-associated inbreeding but also the direction and the magnitude of the inbreeding effect vary over time, also after accounting for the biases that phenotype-associated inbreeding introduces. Differences in the magnitude of inbreeding depression can be caused by environmental variation, for example with stronger inbreeding depression under more adverse conditions and less inbreeding depression under more benign conditions, as for example shown in Darwin's finches (*Geospiza scandens*) and song sparrows (Keller et al. 2002, Charmantier and Garant 2005 for review, Marr et al. 2006). In addition, changes in the sign of inbreeding effects may be mediated by genetic changes over time (Curik et al. 2001).

We have shown phenotype-associated inbreeding in this particular population of white-throated dipper, but how general is this phenomenon? It can arise as a consequence of non-random mate choice, for example if individuals with a particular phenotype either prefer or are forced to mate with kin more often than expected by chance (Reid et al. 2008). More general however, phenotype-dependent dispersal may generate phenotype-associated inbreeding under random mating. Although in many evolutionary genetic models dispersal and gene flow are assumed to be random and thus phenotype-independent (see Lenormand 2002, Räsänen and Hendry 2008), this assumption may be violated in natural populations (Edelaar and Bolnick 2012). For example, dispersing and philopatric individuals may differ in their morphology (e.g. Paradis et al. 1998, Skjelseth et al. 2007, Dawideit et al. 2009), in their personality (e.g. Cote et al. 2011) or in fitness-related traits (e.g. Friedenberg 2003, Innocent et al. 2010). Furthermore, immigrants can differ in traits not directly related to dispersal, but which differ between the population of origin and the receiving population, for example due to local adaptation (e.g. Postma and Van Noordwijk 2005).

Hence, conditions for phenotype-dependent inbreeding are likely to be fulfilled in many cases, in particular in populations living in spatially fragmented environments and when dispersal is phenotype-dependent, and studies of inbreeding depression have to be aware of the potential biases this may introduce. However, it is important to note that the size of this bias is related to the heritability of the trait (i.e. the proportion of phenotypic variance that is explained by additive genetic variance). Although most traits do show additive genetic variance, heritability is usually

lower in fitness-related traits than in morphological traits (Mousseau and Roff 1987, Postma 2014), suggesting that a potential bias will be smaller in the former.

A straightforward approach for avoiding a potential bias in the estimate of inbreeding depression is to simultaneously account for the inbreeding coefficient and additive genetic effects. As we showed here, this is possible within a quantitative genetic animal model framework. Because inbreeding coefficients in individual long-term studies are generally calculated based on the pedigree data, this requires no additional data. Indeed, whereas the importance of including inbreeding coefficients as an additional covariate in the animal model to obtain unbiased estimates of additive genetic variance and heritability has been emphasized repeatedly (Hoeschele and Van Raden 1991, de Boer and Van Arendonk 1992, reviewed in Wolak and Keller 2014), the importance of including additive genetic effects when estimating the effect of inbreeding has so far obtained less attention.

In summary, we have shown phenotype-associated inbreeding in a long-term study population of white-throated dippers. However, patterns varied over time, with inbred individuals having on average longer-winged parents in some cohorts, but shorter-winged parents in other cohorts. When we did not account for additive genetic effects, these patterns of phenotype-associated inbreeding resulted in a substantial under- or overestimation of inbreeding depression, respectively. Because phenotype-associated inbreeding, in particular due to phenotype-dependent dispersal in spatially fragmented environments, is likely to be common, it should ideally be accounted for in future studies of inbreeding depression.

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Chapter 5

Mother-offspring resemblance but no additive genetic variation in telomere length in white-throated dippers

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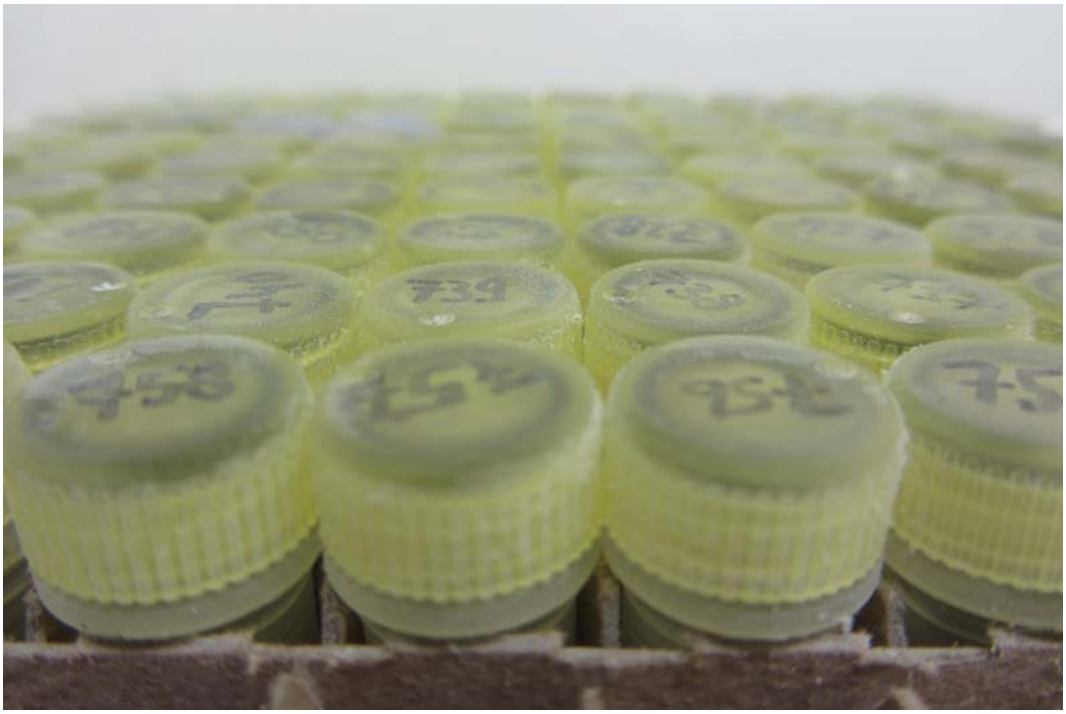

Abstract

Telomeres are protective DNA-protein complexes located at the ends of eukaryotic chromosomes, whose length has been shown to predict life-history parameters in various species. Although this suggests that telomere length is subject to natural selection, its evolutionary dynamics crucially depends on its heritability. Using pedigree data from a population of European dippers (*Cinclus cinclus*), we test whether and how variation in early-life relative telomere length (RTL, measured as the amount of telomere sequences relative to a control gene using qPCR) is transmitted across generations. We disentangle the relative effects of genes and environment, and test for sex-specific patterns of inheritance. Although we find significant resemblance between mother and offspring (and between mother and son in particular) and among offspring sharing the same nest, as well as some indication for an effect of inbreeding, additive genetic variance and heritability are close to zero. We show that neither maternal imprinting nor Z-linked inheritance can explain these patterns of resemblance, suggesting they are due to non-genetic maternal and common environment effects instead. We conclude that environmental factors are the main drivers of variation in early-life RTL in a wild bird population, which will severely bias estimates of heritability when not modeled explicitly.

Keywords: relative telomere length · heritability · maternal effect · sex-linkage · inbreeding · bird

Introduction

Telomeres are highly conserved protective DNA-protein complexes based on tandem repeats of a simple sequence of nucleotides. Although telomeric sequences are typically located at the ends of eukaryotic chromosomes (terminal telomeres) they can also be found in the pericentric regions of chromosomes (interstitial telomeres, Meyne et al. 1990, Foote et al. 2013). Terminal telomeres prevent deterioration of chromosome ends and fusion among chromosomes (Blackburn 2000). Changes in their length depend on the interplay of pro- and anti-erosion factors (von Zglinicki 2000, Blackburn 2001). Telomere length has been shown to significantly predict life-history parameters in a number of organisms, both when telomeres are measured early in life (Lindström 1999, Metcalfe and Monaghan 2001, Heidinger et al. 2012), and during adulthood (Bize et al. 2009). For example, telomere length (or their rate of shortening) has been linked to lifespan in humans (Cawthon et al. 2003) and several bird species, both in captivity (Heidinger et al. 2012) and in natural populations (Haussmann et al. 2005, Bize et al. 2009, Salomons et al. 2009). More recently, it has been found that telomere length may not only be positively related to individual fitness through its link with lifespan, but also as a mediator of reproductive trade-offs (Bauch et al. 2013). This further reinforces the idea that telomere length could be subject to directional natural selection (Fulnečková et al. 2013). However, whether this results also in an evolutionary response depends on the heritability of telomere length.

Telomere length shows substantial amounts of variation, not only among eukaryote species (Forsyth et al. 2002, Gomes et al. 2010), but also among individiduals of the same species and population (Bize et al. 2009). Although it is key to obtaining a better understanding of the origin of this individual variation in early life telomere length, an answer to the questions whether variation in telomere length is transmitted from one generation to the next, and if it is, by what mechanism, remains elusive.

To date, several studies on the mode of transmission and narrow-sense heritability (i.e. the proportion of the phenotypic variance that is attributable to additive genetic effects, h^2) of telomere length have been conducted in humans (e.g. Slagboom et al. 1994, Graakjaer et al. 2004). Estimates from natural populations of other species, however, remain scarce (but see Horn et al. 2011, Olsson et al. 2011, Voillemot et al. 2012). Ranging from 0.18 to 1.23 (Slagboom et al. 1994, Njajou et al. 2007, Olsson et al. 2011, Voillemot et al. 2012), the majority of heritability estimates is relatively high, especially considering that heritabilities of traits that are closely related to fitness are often low (Price and Schluter 1991). However, as they are ratios, heritabilities can be high even if the absolute amount of additive genetic variance is low, if environmentally-induced variation is even lower, as may be the case in captivity (but see

Weigensberg and Roff 1996). Alternatively, if common environment and parental effects are not accounted for, heritabilities will be overestimated (Kruuk and Hadfield 2007). Indeed, telomere dynamics are known to be modulated to a large extent by environmental factors, both during development (Jennings et al. 1999, Tarry-Adkins et al. 2008, Geiger et al. 2012) and adult life (Epel et al. 2004, Monaghan and Haussmann 2006, Blackburn and Epel 2012). For example, early exposure to steroid hormones triggers an accelerated telomere loss in domestic chickens (Haussmann et al. 2012).

Interestingly, a number of studies has found support for sex-specific patterns of inheritance of telomere length (e.g. Nawrot et al. 2004, Nordfjäll et al. 2005, Horn et al. 2011, Olsson et al. 2011, Broer et al. 2013). For example, the few studies investigating telomere length inheritance in non-human animals (birds and lizards) have found either maternal inheritance (Horn et al. 2011) or a much higher son-father than daughter-mother resemblance (Olsson et al. 2011). Indeed, sex-specific patterns appear to be the rule rather than the exception, and suggested mechanisms include parent-specific imprinting, hormonal regulation and sex-chromosome linkage, either acting independently or jointly (Nordfjäll et al. 2005, Horn et al. 2011, Olsson et al. 2011, Broer et al. 2013)

Here, we investigate patterns of inheritance of relative telomere length (RTL) in a wild population of European dippers (*Cinclus cinclus*). We use nestlings from an individual-based long-term study, enabling us to separate phenotypic variation in RTL into variance components attributable to additive genetic, common environment (i.e. nest) and other environmental effects. In addition, we explicitly test for parental and imprinting effects, as well as sex-linked inheritance (Z-linkage).

Materials & Methods

Study system

The European dipper is a medium-sized passerine living along streams and rivers. Since 1987, dippers have been studied at eleven rivers spanning an area of approximately 400 km² in the proximity of Zurich, northern Switzerland (8°23'E / 47°25'N to 8°40'E / 47°10'N). Here, we use data from the Küsnacht (*K)*, Wehrenbach (*W)*, and Sihl *(S)* rivers. Every year, monitoring starts in early February in order to map territories and to find nests. Most pairs are socially monogamous but each year approximately 9% of males are polygynous. Territories are checked regularly between nest building and nestling phase. Nestlings of first broods hatch between early March and April. About 35% of all nestlings are from second broods with hatching dates in early June at the latest. Clutch size is slightly larger for the first compared to the second brood (mean ± s.d.: 4.8 ± 1.0 vs. 4.5 ± 0.9), which is also reflected in the number of nestlings (4.4 ± 1.1 vs. 3.8 ± 1.2). Incubation takes 16-17 days and offspring fledge at an age of 21-24 days. Both parents feed their offspring but only females incubate. When nestlings are 10-14 days old (min. 7 days, max 17 days), they are ringed and a small blood sample (max. 30 µl) is collected by puncturing the tarsal vein. Unringed adults (i.e. immigrants) are captured using mist nets and ringed, usually before the breeding season, but at the latest before their offspring are ringed.

Pedigree reconstruction and inbreeding estimation

Parentage of each brood was determined from behavioural observations, assuming that the social parents are also the genetic parents of a nestling. This is a reasonable assumption given the low incidence of extra-pair paternity in these study populations (less than 1% of nestlings; unpublished data). The identity of the territorial breeding male and female were recorded during territory establishment, incubation and/or feeding of the offspring.

We were able to construct a pedigree spanning 15 generations, covering the cohorts from 1987 to 2012. We calculated Wright's coefficients of inbreeding *f* (Wright 1969) for each individual using *Pedigree Viewe*r (available at http://www.personal.une.edu.au/~bkinghor/ pedigree.htm). Inbreeding coefficients are relative to the base population, i.e. relative to all birds with unknown parents. As a consequence, founders and immigrants are assigned an inbreeding coefficient of zero, which carries no information. Therefore, in the following analyses we use only birds that hatched in the study area.

We selected a total of 177 individuals of the cohorts 2002 to 2011 for RTL measurement, consisting of interconnected groups made up by a sire, a dam and – whenever possible – one female and one male offspring. This study design, which maximizes the number of families rather than the number of individuals per family, will have resulted in a slight reduction in the precision (i.e. the standard error) of our estimate of the (environmental) variance between nests. More importantly however, this also resulted in more independent parent-offspring links, as well as several pedigree links between families: 16 females and 9 males are represented as both offspring and parent in the data set. Furthermore, several individuals have sired offspring with more than one mate (average 1.59 for males and 1.55 for females). Therefore, our dataset also contains pedigree links between grandparents and their grandchildren or between half sibs, etc. Thereby our study design provided relatively accurate and precise estimates of additive genetic variance and heritability. Finally, we deliberately selected a high proportion of inbred individuals to be able to estimate a potential inbreeding effect. As a consequence, the mean inbreeding coefficient (± standard deviation) of the selected individuals was much higher (0.076 ± 0.088; f=0: N=64, 0<f≤0.0625: N=36, 0.0625<f≤0.25: N=62, f>0.25: N=15, max f=0.3037) than the population-level mean for the same cohorts and rivers (0.023 ± 0.057). Pruning the complete pedigree used for the calculation of inbreeding coefficients (see above) to include only individuals with known RTL, or that provide a pedigree link between two individuals with known RTL (using the R package "pedantics", Morrissey and Wilson 2010), resulted in a pedigree containing 315 individuals, 255 maternities, 263 paternities, 221 full sibs, 103 maternal half sibs and 147 paternal half sibs. Mean pedigree depth was 5.9 generations (max. 14 generations).

DNA extraction, storage and sexing

Blood samples were preserved up to several months at approximately 4°C (starting at the day of sampling) in APS buffer (Arctander 1988). DNA was extracted using the QIAmp DNA mini kit (BioSprint 96, Quiagen) and then stored in AE buffer at -20°C. Within a few weeks DNA concentration was normalized and afterwards DNA was stored at -80°C (see Bucher et al. 2009 for more details). Nestling sex was determined by amplifying the CHD-W and CHD-Z genes using modified versions of the P2 and P8 primers (Griffiths et al. 1998, Hoeck et al. 2009).

Relative telomere length (RTL) measurements

RTL was assessed using a quantitative real-time amplification (qPCR) procedure (Cawthon 2002) adjusted for bird species (Criscuolo et al. 2009). Because telomeres have never been studied in European dippers before, we give all necessary details on the measurement methods and thereby follow the guidelines as provided by Nussey et al. (Nussey et al. 2014).

We verified quality and purity of extracted DNA with a NanoDrop (Thermo Scientific) spectrophotometer (absorbance ratio A260/280>1.7; A260/230>1.8). Although qPCR assays do not only amplify terminal telomeric repeats, but also interstitial telomeric sequences near the centromeres (Nakagawa et al. 2004), RTL measurement via qPCR has been validated and successfully applied in a number of bird species (e.g. Bize et al. 2009, Criscuolo et al. 2009, Heidinger et al. 2012, Voillemot et al. 2012). Interstitial repeats may increase the noise in the estimates, thereby making it harder to detect patterns (Foote et al. 2013).

Telomere length was expressed as the ratio of the amount of telomere sequence to the amount for a non-telomeric control gene that is non-variable in copy number in the study species (relative telomere length, RTL). The amount of telomere sequence present in the sample (T), and the amount of the control gene (control gene S) are proportional to the number of qPCR amplification cycles needed to reach a threshold fluorescent signal (C_q value) in the exponential growth phase. Here we used glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Genbank Accession No: AF255390) as the control gene. To validate both the expected amplicon size of the control gene (50 bp) and its uniformity in European dippers, we performed a standard electrophoresis on a 1.5% agarose gel run in TBE buffer with 14 randomly chosen samples (see Fig S1).

Forward and reverse primers for the GAPDH gene were 5'-AACCAGCCAAGTACGATGACAT-3' and 5'-CCATCAGCAGCAGCCTTCA-3' respectively. Primers for amplification of telomere sequences were 5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3' and 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3' (Tel1b and Tel2b, see Criscuolo et al. 2009). qPCRs for both telomere sequences and GAPDH were performed using 5 ng of DNA with both sets of primers, in a final volume of 10 µl containing 5 µl of Power SYBR© Green PCR Master Mix (Appliedbiosystems, UK). Primer concentrations in the final mix were 100 nM for the telomere assay and 200 nM for the control gene assay. Real time amplification of telomere sequences and GADPH were performed on separate 96-well plates. qPCR conditions for amplification of telomere sequences were 10 min at 95°C, followed by 30 cycles of 1 min at 56°C and 1 min at 95°C. qPCR conditions for GAPDH amplification were 10 min at 95°C, followed by 40 cycles of 1 min at 60°C and 1 min at 95°C.

Samples were randomly assigned to one of six plates. All samples, including both reference samples and dilution series (see below), were analysed in duplicate. The precision of qPCR measurements critically depends on amplification efficiencies (Smith et al. 2011). In order to control for variation in the amplification efficiency of the qPCR among plates, serial dilutions (50ng, 10ng, 2ng, 0.4ng, 0.08ng, 0.016ng) of a reference sample were used to generate a reference curve for each plate. Both a negative control (water) and a melting curve were run for each plate to check for specific amplification of a unique amplicon and for the absence of primer-dimer artefact (Fig S2).

Intra-plate mean coefficients of variation for C_q values were 1.35 ± 0.06% for the telomere assay and 0.79 ± 0.04% for the control gene assay (based on duplicates), and inter-plate coefficients of variation based on repeated samples (n=5) were 1.56% for the telomere assay and 1.35% for the control gene assay (all CV calculated before correction for plate effects). Amplification efficiencies (estimated from the standard curves of serial dilutions) of the qPCR runs were between 98% and 100% for telomeric repeats and between 99% and 100% for the control gene. To take into account both this slight difference in amplification efficiency (E), as well as the non-zero intra- and inter-plate coefficients of variation, we calculated relative telomere length following Pfaffl (Pfaffl 2001) as

$$\text{relative telomere length} = \frac{1 + \mathrm{E} \cdot \mathrm{T}^{\Delta C_q \,\mathrm{T}\cdot(\text{control}-\text{sample})}}{1 + \mathrm{E} \cdot \mathrm{S}^{\Delta C_q \,\mathrm{S}\cdot(\text{control}-\text{sample})}}$$

The coefficient of variation for relative telomere length was 12.7%.

Quantitative genetic analyses

We fitted a series of animal models (Kruuk 2004) to estimate the absolute and relative amount of additive genetic variance (V_A and h^2, respectively) underlying telomere length, and to test for parental effects, sex-specific inheritance and imprinting. Animal models were fitted using restricted maximum likelihood (REML) in ASReml version 3.0 (Gilmour et al. 2009), except for models including imprinting effects, which were implemented in WOMBAT (Meyer 2007).

RTL was best described by a normal distribution and residuals of the final model did not show any deviations from normality (Shapiro-Wilk test of normality, W=0.99, p=0.25). Nestling RTL was modelled as a function of sex and natal population (*i.e.* river), age at sampling (in days), and

hatching date (as Julian day). Because within-brood competition might act as a stressor which might negatively affect RTL, we fitted brood size, as well as body mass and tarsus length as proxies for body condition, as covariates. Tarsus length and body mass were included as residual values from a quadratic regression that accounts for age effects during nestling growth. Additionally, all models included the inbreeding coefficient as a covariate, which in the presence of inbreeding depression in RTL ensures unbiased estimates of additive genetic variance (Hoeschele and van Raden 1991, de Boer and van Arendonk 1992, reviewed in Wolak and Keller 2014). Fixed effects were removed in a stepwise manner, starting with the least significant, as inferred from a conditional Wald F-test. All effects with $p<0.15$ were retained in the model.
In addition to the fixed effects listed above, we fitted a random additive genetic effect (animal effect), as well as a random nest and year of birth effect. The animal effect (V_A) estimates the variance in the trait that is due to additive genetic effects, using information on the relatedness and resemblance in telomere length among all individuals in the pedigree. The nest effect (V_{NEST}) estimates the variance among nests that can be attributed to the shared environment of full sibs growing up in the same nest, over and above the variance that is attributable to additive genetic effects. Finally, variation that can be attributed to random environmental variability among years (V_{YEAR}) is accounted for by the year of birth effect. Heritability, the proportion of the phenotypic variance that is explained by additive genetic variance, was calculated as

$$h^2 = V_A / (V_A + V_{NEST} + V_{YEAR} + V_R),$$

where V_R is the residual variance. Statistical significance of random effects was assessed using likelihood ratio tests, comparing log-likelihoods of models with and without the specific random effect.

To test whether there are general features of the mother or father that affect offspring RTL over and above any additive genetic effects that she or he passed on (sensu Willham 1972), initial models included maternal and paternal identity as additional random effects. However, both explained little to no variation (2.6×10^{-5} % and 3.1×10^{-5} % of phenotypic variation, respectively). Hence, parental identities were excluded from any further models.

Although the structure of the data did not allow us to unequivocally attribute the increased resemblance among full sibs to properties of the nest or the mother, we were able to directly test for an effect of maternal RTL (as a nestling) on the RTL of her offspring, again over and above the effect of the genes that she passes on to them. To do so, we extended the animal model arrived at above with maternal RTL (m, after correcting for the effects outlined above) as an additional

covariate, as outlined in Lynch and Walsh (page 706 in Lynch and Walsh 1998). Similarly, we included residual paternal RTL, and also both maternal and paternal RTL simultaneously.

In birds, females are the heterogametic and males the homogametic sex (ZW and ZZ, respectively). Animal models allow for the explicit estimation of sex-linked effects as these follow a different pattern of inheritance than autosomal traits (Gilmour et al. 2009, Husby et al. 2012). In order to quantify Z-linked genetic variance, we used a relatedness matrix that accounts for the specific inheritance of Z-linked genes (e.g. the relatedness between mothers and their daughters is zero) (Husby et al. 2012) instead of the usual autosomal relatedness matrix.

Finally, we tested for imprinting effects, which may provide a further source of sex-specific resemblance, using WOMBAT (Meyer 2007). We externally calculated the inverse of a gametic relationship matrix (code written by Bruce Tier, provided on http://didgeridoo.une.edu.au/womwiki) and used this to fit either a random maternal or a paternal imprinting effect, in addition to the animal's additive genetic effect.

In addition to the animal models above, we performed a number of parent-offspring regressions, in which we regressed mean offspring, son or daughter RTL against maternal or paternal RTL. Values that were used for these regressions were residuals taken from a mixed model that accounts for the effects of inbreeding coefficient and year of birth. Not only do these help to visualise our main findings, they also make it possible to directly compare them to other studies on parent-offspring resemblance in RTL.

Results

Animal model analyses

RTL was not affected by hatching date (b=2.5 x 10^{-4} ± 1.5 x 10^{-3}, $F_{1,102.6}$=0.03, p=0.87), age at sampling (b=1.4 x 10^{-3} ± 1.7 x 10^{-2}, $F_{1,95.4}$=0.01, p=0.94), sex (male-female length: 1.4 x 10^{-2} ± 4.1 x 10^{-2}, $F_{1,102.8}$=0.11, p=0.74), or natal population ($F_{2,93.1}$=1.74, p=0.18). Similarly, brood size (b=-2.7 x 10^{-2} ± 2.7 x 10^{-2}, $F_{1,101.5}$=0.97, p=0.33), age-corrected body mass (b=4.0 x 10^{-3} ± 4.6 x 10^{-3}, $F_{1,163.4}$=0.73, p=0.40) and tarsus length (b=-1.3 x 10^{-3} ± 1.8 x 10^{-2}, $F_{1,121.3}$=0.01, p=0.86) did not predict early-life RTL. Although it did not reach statistical significance, inbreeding had a positive effect on RTL (b=0.53 ± 0.35, $F_{1,64.0}$=2.29, p=0.14), and was retained in all models to obtain unbiased variance component estimates (Hoeschele and van Raden 1991, de Boer and van Arendonk 1992).

Nest identity and year of birth explained large and significant proportions of the phenotypic variation (± approximate standard error) (nest identity: 19.6 ± 8.3%, χ^2=8.59, d.f.=1, p=0.002; and year of birth: 45.7 ± 13.2%, χ^2=52.06, d.f.=1, p<0.001). However, the additive genetic variance was not significantly different from zero (0.007 ± 0.013, χ^2=0.45, d.f.=1, p=0.25), and heritability of RTL was estimated to be 3.8 ± 6.9%. For all statistical details, see Table 1.

Including residual maternal RTL resulted in a smaller sample size (n=114) and in a further reduction of V_A (1 x 10^{-7}, χ^2=0, d.f.=1, p=0.50) and h^2 (6 x 10^{-5}%), but did not affect estimates of the other variance components (Table 1). Again, the effect of inbreeding was positive and this time did reach statistical significance (b=0.87 ± 0.43, $F_{1,46.8}$=4.14, p=0.05). Most importantly, maternal RTL significantly explained variation in offspring RTL (b=0.22 ± 0.11, $F_{1,47.1}$=4.06, p=0.048; Table 1). Although the additional inclusion of paternal RTL further reduced the sample size (n=101), this did not alter the positive point estimate for the effect of the mother's phenotype on offspring phenotype (b=0.23 ± 0.12, $F_{1,39.4}$=3.35, p=0.075). Although there was no effect of the father's phenotype (b=0.02 ± 0.13, $F_{1,37.9}$=0.04, p=0.85), it did not differ significantly from the effect of maternal RTL (interaction term: $F_{2,38.9}$=0.88, p=0.35).

Additional analyses attempting to explain these patterns of resemblance by means of sex-linked inheritance revealed a random variance component for Z chromosome-linked variance of 5.3x10^{-8} (χ^2=0, d.f.=1, p=0.50), with all other variance component estimates remaining unchanged. Similarly, neither maternal nor paternal imprinting explained much variance (maternal imprinting 1.6 x 10^{-5}, χ^2=0, d.f.=1, p=0.50; paternal imprinting: 1.2 x 10^{-5}, χ^2=0, d.f.=1, p=0.50).

Table 1*:* Animal model analysis, explaining variation in nestling telomere length with an individual's inbreeding coefficient (f) as well as an additive genetic effect (animal effect), a nest and a year of birth effect (model A, n=177). Model B (n=114) additionally includes maternal residual telomere length (TL) as a covariate. Slopes for covariates, variance components for random effects as well as their proportions are given including approximate standard errors (s.e.) and test statistics (conditional F-test and χ^2-test, respectively).

	model A				**model B**			
covariate	**slope ± s.e**		**test statistic**	**p-value**	**slope ± s.e**		**test statistic**	**p-value**
f	0.53 ± 0.35		$F_{1,64.0}$=2.29	0.14	0.87 ± 0.43		$F_{1,46.8}$=4.14	0.05
maternal TL	–		–	–	0.22 ± 0.11		$F_{1,47.1}$=4.06	0.048
random effect	**variance ± s.e.**	**proportion ± s.e.**	**test statistic**	**p-value**	**variance ± s.e.**	**proportion ± s.e.**	**test statistic**	**p-value**
animal (V_A)	0.007 ± 0.013	0.038 ± 0.069	χ^2=0.45	0.25	1×10^{-7}	$6x10^{-7}$	χ^2=0	0.5
nest (V_{NEST})	0.037 ± 0.014	0.196 ± 0.083	χ^2=8.59	0.002	0.031 ± 0.015	0.182 ± 0.092	χ^2=5.77	0.008
year of birth (V_{YEAR})	0.086 ± 0.044	0.457 ± 0.132	χ^2=52.06	<0.001	0.075 ± 0.045	0.438 ± 0.152	χ^2=23.71	<0.001
residual (V_R)	0.058 ± 0.012	0.310 ± 0.096			0.066 ± 0.012	0.038 ± 0.118		

Parent-offspring regressions

The slope of the regression of mid-offspring on maternal RTL was significantly positive (b=0.22 ± 0.10, p=0.03; n=59), whereas the slope of the regression of mid-offspring on paternal RTL was small and non-significant (b = 0.04 ± 0.11, p=0.73; n=59) (Fig. 1, Table 2). Again, the two estimates of the regression slopes were not significantly different from each other (t=1.21, p=0.23). Similarly, single sex offspring – parent regressions revealed that daughter-father and son-father regressions were not significantly different from zero, with estimates close to zero (b=0.09 ± 0.14, p=0.50, and b=–0.03 ± 0.12, p=0.79, respectively). However, single sex offspring-mother regressions showed that resemblance between sons and their mothers (b=0.27 ± 0.04, p=0.01) was considerably but not significantly (t=1.12, p=0.27) higher than the resemblance between daughters and their mothers (b=0.11 ± 0.14, p=0.43).

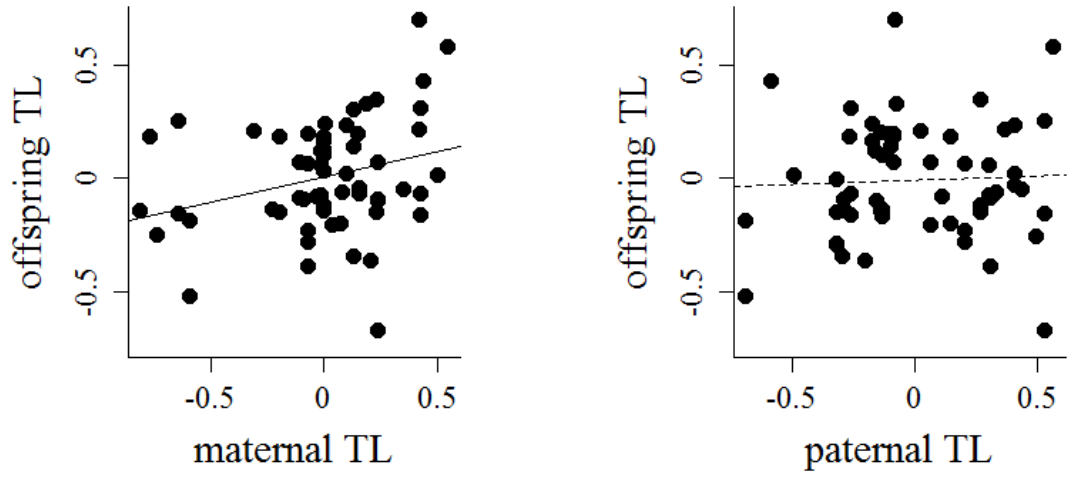

Figure 1: Linear regressions of mid-offspring telomere length (TL; mean of nestlings of one nest) against their mother's (left panel, n=59) and father's (right panel, n=59) telomere length. TL values are residuals from a mixed model accounting for an individual's inbreeding coefficient and its year of birth. Whereas offspring resemble their mother (b=0.22 ± 0.10, p=0.03), there is no correlation between fathers and their offspring (b=0.04 ± 0.11, p=0.73).

Table 2: Parent-offspring regressions of telomere length. Slope including standard error (b ± s.e.), sample size (n) and p-value are given for different regressions of offspring (mid-offspring, son, or daughter) on parental telomere length. Values of telomere length are residuals from a mixed model that accounts for an individual's inbreeding coefficient and its year of birth. All telomere measurements were done on samples taken at the nestling stage.

parent – offspring combination	n	b ± s.e	p-value
mother – offspring	59	0.22 ± 0.10	0.03
mother – son	58	0.27 ± 0.04	0.01
mother – daughter	56	0.11 ± 0.14	0.43
father – offspring	59	0.04 ± 0.11	0.73
father – son	61	-0.03 ± 0.12	0.79
father – daughter	57	0.09 ± 0.14	0.50

Discussion

Here we tested whether and how variation in RTL is transmitted across generations. By using pedigree data from a wild population of European dippers, we were able to disentangle the relative effects of genes and the environment on early life RTL, and to test for sex-specific patterns of inheritance.

Previously reported heritability estimates of telomere length range between 0.44 and 0.78 for humans (Slagboom et al. 1994, Njajou et al. 2007, Broer et al. 2013) and between 0.18-1.23 for other vertebrates (Horn et al. 2011, Olsson et al. 2011, Voillemot et al. 2012). If estimates of heritability are based on offspring-parent regressions, potential variance components of a common environment might remain unaccounted for, resulting in an overestimate of heritability (Kruuk 2004). Indeed, based on a mother-offspring regression, we would have obtained a statistically significant and relatively high heritability of 44% (twice the slope of the mother-offspring regression), even after accounting for the effects of year of birth and inbreeding coefficient. Similarly, not accounting for nest effects in our animal model would have resulted in a heritability of 9.2%, whereas accounting for nest effects reduces heritability to 3.8% (± 6.9%). The latter is in line with a cross-fostering experiment in a wild population of collared flycatchers (*Ficedula albicollis*), which in principle allows for the separation of additive genetic and common environment effects, and which found a heritability of 18% (and not 9% as reported previously (Bize, pers. comm., Voillemot et al. 2012)). Although this value is higher than our estimate in dippers, it still is substantially lower than what has been found in other studies. Furthermore, it should be noted that the nature of their experimental design and statistical analyses still allows for a substantial inflation of the heritability (Voillemot et al. 2012).

Although we find no evidence for autosomal additive genetic variance underlying variation in RTL, we do find a significant link between offspring and maternal RTL, and in particular between mothers and their sons, whereas we find no such link between offspring and paternal RTL. Such parental effects have been found previously in humans and birds (e.g. Horn et al. 2011, Broer et al. 2013). For example, Horn *et al.* (Horn et al. 2011) found patterns of parent-offspring resemblance in kakapos (*Strigops habroptilus*) that are strikingly similar to our results: whereas mothers had similar telomere lengths as their offspring, in particular when compared with their sons, offspring did not show any resemblance to their father (Horn et al. 2011). Sand lizard males on the other hand, show a higher resemblance to their father than to their mother (Olsson et al. 2011).

One mechanism that could explain such sex-specific patterns is sex-specific gene imprinting. However, we found no evidence of either maternal or paternal imprinting. Similarly, we found no evidence for sex-linkage, specifically Z-linkage, as an explanation for the sex-specific patterns of resemblance. Furthermore, as the W-chromosome is only transmitted to daughters, the strong resemblance between mothers and their sons rules out W-linkage. In conclusion, we therefore propose that the mother-offspring resemblance observed here is the result of a non-genetic maternal effect. However, we cannot explain the (non-significant) difference in resemblance between mothers and sons or daughters, respectively.

In accordance with previous studies, we find a major role for environmental effects in shaping variation in RTL (Jennings et al. 1999, Hall et al. 2004, Tarry-Adkins et al. 2008, Foote et al. 2011, Geiger et al. 2012, Mizutani et al. 2013, Young et al. 2013). For example, a substantial amount of variation was attributable to environmental differences among birth years (i.e. cohort effects). Similarly, Mizutani et al. (Mizutani et al. 2013) showed for black-tailed gulls (*Larus crassirostris*) that the rate of change in telomere length mainly differed with respect to year, and attributed this to the consequences of El Niño events and the Great Japan Earthquake on food availability.

Although increased telomere shortening in response to suboptimal environmental conditions has been observed in mammals, lizards and birds (Haussmann and Marchetto 2010, Ballen et al. 2012, Young et al. 2013), in our case it remains unclear which environmental variables are responsible for the observed annual variation in early-life RTL. For example, there was no effect of either spring temperature or rainfall on mean RTL (analyses not presented). Furthermore, there were positive correlations among mean RTL, mean nestling body mass and the mean probability of a nestling to produce offspring later in life on the cohort-level, i.e. cohorts with on average higher body mass also had on average longer telomeres. However, within cohorts only the correlation between nestling weight and the probability of producing offspring later in life remained and approached statistical significance ($p=0.06$). This suggests that between- rather than within-year environmental variation shapes both RTL and, for example, body mass, and it argues against a direct relationship between the two.

In addition to population-level environmental year effects, the micro-environment of the nest was an important determinant of early life RTL. This suggests that the main parameters that determine nest microclimate, temperature, humidity, and gas composition (Deeming 2002) may modulate telomere shortening. Indeed, early development is a period characterised by rapid telomere shortening (Hall et al. 2004). For example in captive zebra finches, telomere shortening was six times faster during the first 30 days of growth when compared to the following 550 days of life

(F. Criscuolo unpublished data). Faster telomere shortening early in life has also been demonstrated in the wild, e.g. in jackdaws (*Corvus monedula*) (Salomons et al. 2009). An additional factor which may contribute to nest micro-environment is parental quality. High-quality parental investment may buffer stressful events during early development and preserve telomeres from adverse stress-related weakening, causing significant nest effects. Similarly, Andrew *et al.* (Andrew et al. 2006) showed that 49% of the variation in telomere length could be attributed to (environmental) family effects in a human twin-study.

We found RTL to be positively related to an individual's inbreeding coefficient, which is in line with findings in laboratory mouse strains (Manning et al. 2002) and domesticated chicken lines (O'Hare and Delany 2009). This positive relationship is at first sight surprising, as longer telomeres have been associated with increased survival rate and lifespan (Bize et al. 2009, Heidinger et al. 2012), whereas survival and lifespan are typically lower in inbred individuals (for review see Keller and Waller 2002). Hence, one would intuitively expect telomeres to be shorter in inbred individuals. However, inbred dippers in our population do not have decreased survival rates and lifespan (Becker *et al.*, unpublished data), and in general little is known about the relationship between telomere length and other fitness-related traits such as fecundity (but see Bauch et al. 2013). Indeed, the longer telomeres of inbred nestlings may be a by-product of inbreeding effects on other traits. For example, inbred dipper nestlings are smaller at the age of ringing and blood sampling, indicating slower growth rates (Becker *et al.*, unpublished data). Slower growth rates might entail lower cell division rates, causing less telomere loss due to cell division. However, as body mass or tarsus length (corrected for age at sampling) are not predicting RTL we are not able to further support this hypothesis. Alternatively, the positive link between RTL and inbreeding coefficient may be the product of suppressive effects of inbreeding on certain genes regulating telomere length. For instance, if not expressed, factors such as tumor suppressor genes (BRCA1, French et al. 2006) or DNA end-binding proteins (Ku86, Bailey et al. 1999) could lead to telomere elongation. Further work focusing on the interplay between telomere length, inbreeding and fitness is warranted.

In summary, we here demonstrate significant mother-offspring resemblance, and mother-son resemblance in particular, in early life RTL in a bird species in the wild, but at the same time show that its heritability is very low. As we show that neither maternal imprinting nor Z-linked inheritance contribute to this pattern, we conclude that this resemblance is due to a non-genetic maternal effect. In line with its very low heritability, non-genetic environmental factors are the main drivers influencing early life RTL in dippers.

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Authors' contribution

SR, PB, FC, SM and LK planned and designed the study. JH and PB collected samples, SZ measured telomere length, PB and EP did the statistical analysis, and PB, SR, EP, FC, SM and LK interpreted the results and wrote the manuscript.

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Appendix

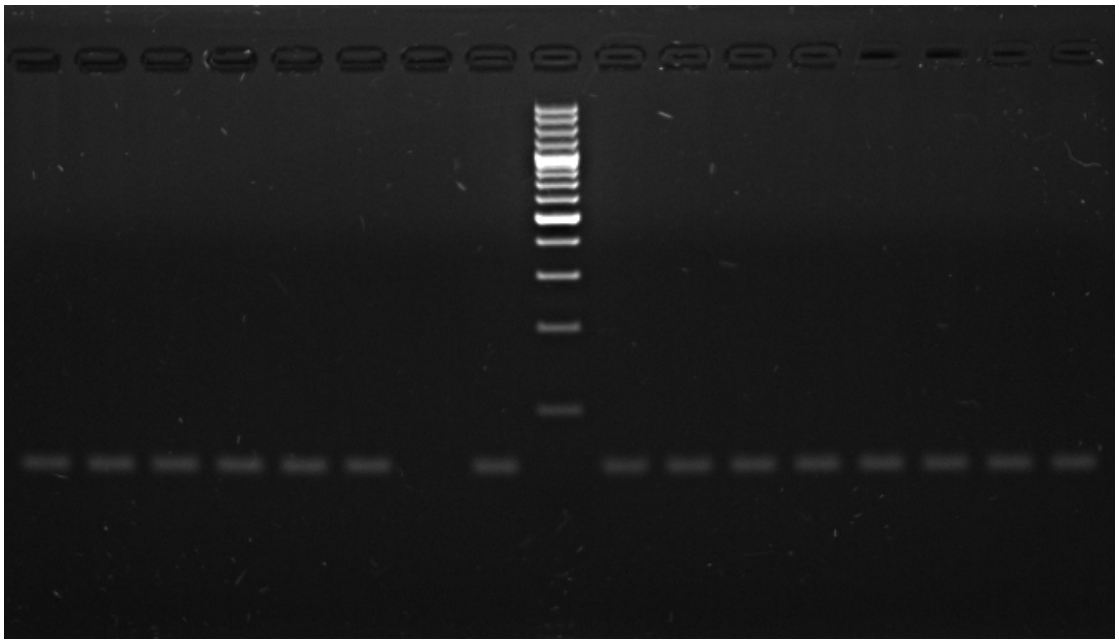

Figure S1: Standard electrophoresis on a 1.5% agarose gel run in TBE buffer of the amplified GADPH (glyceraldehyde-3-phosphate dehydrogenase) product. Electrophoresis was performed for 14 randomly chosen samples to verify the uniformity of the GADPH amplicon over samples. N: negative control; S: sample from standard curve; L: size ladder. The arrow indicates the position of the amplicons at approximately 50 bp.

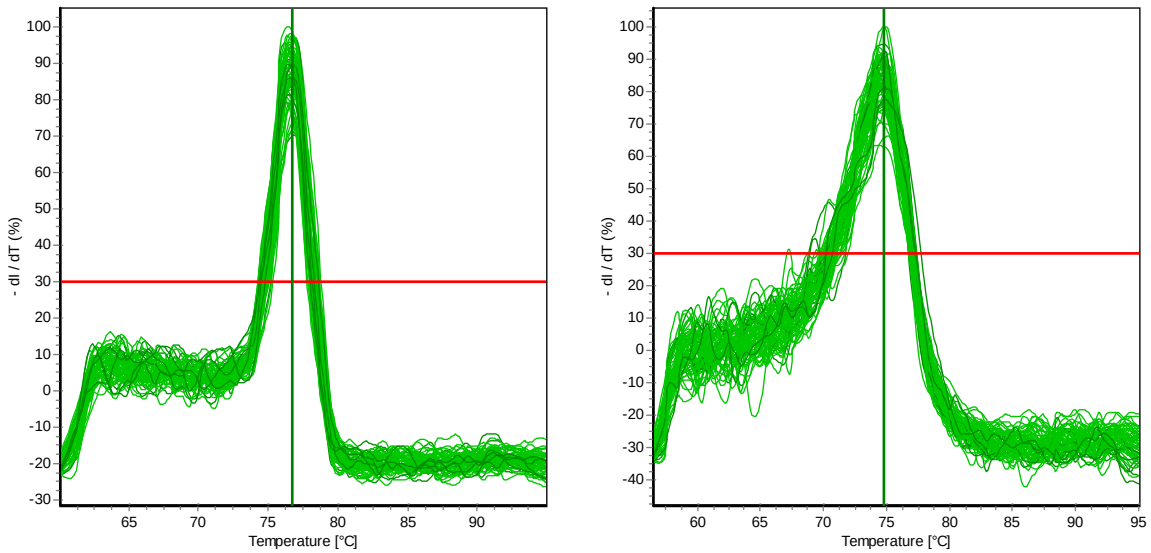

Figure S2: Melting curves of the qPCR assay amplifying the GADPH sequence (left) and telomeric repeats (right). The melting curves of the GADPH assay verify the specific amplification of a unique amplicon and the absence of primer-dimer artefacts. Melting curves of the telomere assay show the absence of primer-dimer artefacts.

Chapter 6

General discussion and perspectives

Summarizing discussion

Nowadays, many species live in fragmented environments. Fragmentation of habitat can be natural (e.g. islands, mountaintops, lakes or rivers) or driven by anthropogenic activities. With populations becoming smaller and more isolated from each other, they have an increased risk of extinction (Smith and Keller 2006). This highlights the importance of studies on species living in fragmented environments, not least because these include many species of conservation concern. The white-throated dipper is a bird species that lives in a naturally fragmented riverine environment. Using a unique long-term data set on this species allowed me to investigate important aspects of dispersal behaviour and genetic variation.

Dispersal is one of the most important life-history traits, being of relevance for many ecological and evolutionary processes (Clobert et al. 2001, Clobert et al. 2012). The outcome of dispersal is often described in terms of distances and rates. However, we still know little about the spatiotemporal properties of the dispersal process itself due to the difficulties of following single individuals precisely over extended periods of time. Capitalizing on the excellent possibilities of observing dippers in their natural environment, I used two years of weekly mark-resight data to study spatiotemporal aspects of dispersal behaviour (Chapter 2). Based on within-river movement data I illustrated emigration from the natal site, a highly mobile transient phase, and the process of settlement. Based on presence-absence data I detected patterns of temporary emigration from the natal river. I conclude that exploratory behaviour during the transient phase is important for finding territories and mates, irrespective of whether individuals settle in their natal or a different population. If temporary emigration is an important component of dispersal behaviour in fragmented environments (see also Reed et al. 1999, Conradt et al. 2001, Doerr and Doerr 2005), increasing degrees of isolation between habitat fragments might affect survival during dispersal and thus shape its evolution. My findings suggest that movement data can also be used to study patterns of settlement and mate choice by specifying visited territories and all opposite-sex individuals in close proximity. Correctly specifying potential mates is necessary for formulating appropriate null models in the analysis of mate choice (Pärt 1996, Szulkin et al. 2013). For example, mate choice can allow the avoidance of inbreeding through kin recognition (see Szulkin et al. 2013). Alternatively (or in addition), dispersal is considered as an inbreeding avoidance mechanism (Hamilton and May 1977, Gandon and Michalakis 2001, Guillaume and Perrin 2006).

Understanding the evolutionary link between dispersal, inbreeding and its avoidance requires data on dispersal and how it shapes the occurrence of inbreeding. In chapter 3, I showed that dispersal in white-throated dippers is female-biased and typically over short distances, but nearly half of all individuals disperse among rivers. In line with this, genetic (microsatellite) data revealed only

weak genetic differentiation between rivers, even on a large spatial scale, but substantial levels of genetic structure on the small (within-river) scale (see also Postma et al. 2009 for a similar finding). Inbreeding occurred frequently due to small population sizes and the linear habitat structure. Furthermore, probabilities of inbreeding in philopatric individuals (i.e. individuals breeding anywhere in their natal river) were higher for females, in particular for dispersal over very short distances. Female-biased probabilities of inbreeding (see also Szulkin and Sheldon 2008) are a consequence of female-biased dispersal but are likely to also maintain this mode of sex-biased dispersal. In conclusion, I argue that weak genetic differentiation among populations does not exclude the frequent occurrence of inbreeding within populations, in particular in small populations of species living in fragmented habitats.

The frequent occurrence of inbreeding raises the question about the consequences of inbreeding. In order to quantify the effects of inbreeding, individual phenotypic values are typically regressed on inbreeding coefficients, while accounting for confounding covariates like age, sex or year. This standard method assumes that inbreeding individuals are a random subsample of the population with respect to the trait of interest (Lynch and Walsh 1998, Reid et al. 2008). Taking wing length in dippers as an example, I tested whether a violation of the above mentioned assumption can bias estimates of inbreeding effects (Chapter 4). I showed that inbreeding individuals were not always a random subsample of the population. During part of the study period, parents of inbred birds had shorter wings than those of outbred birds, and because wing length is heritable, inbred individuals were smaller, independent of any inbreeding effects. This resulted in the overestimation of inbreeding effects. Similarly, during a period when parents of inbred birds had longer wings, I found that inbreeding effects were underestimated. Based on my results I emphasize the importance of simultaneously accounting for inbreeding and additive genetic effects and demonstrate how unbiased estimates of inbreeding depression can be obtained within a quantitative genetics framework.

This is of general relevance as phenotype-associated inbreeding is likely to be common in other systems, too, in particular in spatially fragmented environments. It can arise as a consequence of non-random mate choice, for example if individuals with a particular phenotype either prefer or are forced to mate with kin more often than expected by chance (e.g. Richardson et al. 2004, Reid et al. 2008). More generally however, phenotype-dependent dispersal (e.g. Paradis et al. 1998, Skjelseth et al. 2007, Innocent et al. 2010, Cote et al. 2011) may generate phenotype-associated inbreeding even under random mating because dispersers are less likely to inbreed than philopatric individuals (see Chapter 3 and Szulkin and Sheldon 2008). However, it is important to note that the magnitude of a potential bias is related to the heritability of the trait (i.e. the

proportion of phenotypic variance that is explained by additive genetic variance). Although most traits do show additive genetic variance, heritability is usually lower in fitness-related traits than in morphological traits (Mousseau and Roff 1987, Postma 2014), suggesting that a potential bias will be smaller in the former.

Telomere length is an example for an important trait that has been shown to be linked to fitness-related parameters (Haussmann et al. 2005, Bize et al. 2009, Heidinger et al. 2012). Although this suggests that telomere length could be subject to natural selection, an evolutionary response depends on the heritability of telomere length, which has only been estimated in very few wild populations. Using measures of early-life telomere length in dippers, I showed that additive genetic variance and heritability were close to zero despite significant resemblance between mother and offspring (and between mother and son in particular) and among offspring sharing the same nest (Chapter 5). I showed that neither maternal imprinting nor Z-linked inheritance can explain these patterns of resemblance, suggesting they are due to non-genetic maternal and common environment effects instead. I conclude that environmental factors are the main drivers of variation in early-life telomere length in dippers, which will severely bias estimates of heritability when not modeled explicitly. Ranging from 0.18 to 1.23 (e.g. Horn et al. 2011, Olsson et al. 2011, Voillemot et al. 2012), previous estimates of heritability of telomere length are relatively high for a trait that is supposed to be related to fitness (see above). However, as they are ratios, heritabilities can be high despite low additive genetic variance. This might be the case if environmentally-induced variation is even lower, as expected in captivity (cf. Weigensberg and Roff 1996). Alternatively, if common environment and parental effects are not accounted for, heritabilities will be overestimated (Kruuk and Hadfield 2007). Given that telomere dynamics are known to be modulated to a large extent by environmental factors (e.g. Tarry-Adkins et al. 2008, Haussmann et al. 2012, Mizutani et al. 2013), heritability is likely to be low in the wild, allowing only for weak response to selection.

Perspectives

In the data chapters of this thesis, I used various approaches to obtain new insights into aspects of dispersal and genetic variation, based on a long-term individual-based data set of white-throated dippers. Future research can hopefully build on these findings and address new questions, both in white-throated dippers and others species.

Using a Bayesian multistate mark-recapture analysis I detected signs of temporary emigration from the natal population during the transient phase of the dispersal process. However, mark-

resight data from a single population lack information on the whereabouts of individuals, which have emigrated either temporarily or permanently. That means we do not know whether juveniles explore several populations or only one population, once or repeatedly, or whether individuals have explored their future breeding site or population already earlier and thus possibly compared it to other potential sites or populations. More research on the properties of exploratory behaviour might contribute to our understanding of why individuals remain in or leave their natal population. This might change our view of mortality costs during the transient phase of dispersal. Numerous on-going studies on other species using radio or satellite telemetry techniques will generate large data set that can hopefully answer some of these questions.

As explained above, spatiotemporal data of the dispersal process can reveal insights into patterns of mate choice and enable specifying potentially available mates. Appropriately classifying the latter is necessary for formulating null models of random mating to, for example, detect inbreeding avoidance (see e.g. Pärt 1996, Szulkin et al. 2009, Szulkin et al. 2013). This might allow for separating the effects of dispersal and other mechanisms like kin recognition on inbreeding avoidance. Furthermore, it might provide insights into patterns of phenotype-associated inbreeding (see Chapter 4; and Kruuk et al. 2002, Richardson et al. 2004, Reid et al. 2008).

Given that phenotype-associated inbreeding is likely to also occur in other systems, I emphasized the need for analysing inbreeding depression within a quantitative genetic framework (Chapter 4). Future work on our white-throated dipper study system should include the estimation of inbreeding effects along a life-history continuum (see Szulkin et al. 2007), using animal models. Relevant traits range from hatching rate and nestling survival via juvenile survival and recruitment success to reproductive success and adult survival. For example, I found inbreeding depression on life-time reproductive success and adult lifespan to be non-significant and low without accounting for additive genetic effects (data not shown). However, heritability of fitness-related traits is predicted to be low (Mousseau and Roff 1987, Postma 2014) and philopatric and immigrant dippers do not differ in these traits (data not shown). Thus, inbreeding depression is likely to remain weak, even after accounting for additive genetic effects. As inbreeding has been shown to have negative consequences on individual performance in many species (Charlesworth and Charlesworth 1987, Keller and Waller 2002), it remains exiting to understand why inbreeding depression is probably weak in this system. In this context, it should be emphasized that dippers evolved in a naturally fragmented environment with inbreeding occurring frequently. A potential mechanism to reduce inbreeding depression is purging, i.e. the selective removal of deleterious recessive mutations, when they are exposed in inbred individuals. However, against the

background of high levels of gene flow between populations (Chapter 3), purging becomes an unlikely scenario, in particular when inbreeding is due to genetic drift or populations are small (Glemin et al. 2006, Boakes et al. 2007).

High gene flow between populations will not only result in weak neutral genetic differentiation but can likewise shape quantitative genetic variation, depending on the strength of selection (Postma and Van Noordwijk 2005). For example, in dippers laying date is strongly correlated with water temperature (Hegelbach 2013). Water temperature is a function of altitude and is additionally influenced by air temperature and the habitat surrounding the river (e.g. forested areas vs. urban environments). Therefore, water temperature is an environmental variable that does not only vary in space but also in time due to climate change. Phenotypic responses might thus be driven by evolutionary change and/or phenotypic plasticity (Pigliucci 2005, Charmantier and Gienapp 2014). Long-term data sets offer unique opportunities to study how gene flow and environmental change shape genetic and phenotypic variation, for example in laying date, and to disentangle the effects of evolutionary change and phenotypic plasticity.

Likewise, dispersal behaviour might be under selection, with the response to selection depending on its heritability (see e.g. Thomas et al. 2001). However, estimating the heritability of dispersal behaviour requires the explicit modelling of confounding sources of variation. First estimates of heritability of dispersal were based on parent-offspring correlations (Greenwood et al. 1979). However, it was argued that such correlations can simply emerge from the spatial characteristics of study areas of limited size (Van Noordwijk 1984). Studies finding strong condition-dependence of dispersal suggest that its heritability is low (Ims and Hjermann 2001). In contrast, empirical studies on great reed warblers and collared flycatchers report heritabilities between 0.3 and 0.5 (Hansson et al. 2003, Doligez et al. 2009). A recent study, analysing heritability of (local, i.e. within study area) dispersal distance of great tits using a quantitative genetics framework, reports a heritability estimate of 0.15 (Korsten et al. 2013). In any case, it seems necessary to compare results with estimates from simulated data sets, in which dispersal is governed by non-genetic factors only. Such an approach will show the effects of the spatial characteristics of study areas on estimates of “heritability” and allow the interpretation of estimates from empirical data. Though challenging to obtain, estimates of heritability will proof valuable and important for predicting the evolution of dispersal, in response to both increasing habitat fragmentation and climate change (Davis and Shaw 2001, Thomas et al. 2001, Thomas et al. 2004, Kokko and López-Sepulcre 2006, Hellmann et al. 2008, Ahlroth et al. 2010).

Insights of this thesis and future research on related topics will hopefully contribute to a better understanding of the eco-evolutionary dynamics of species living in fragmented environments.

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